Definition of the ACGT clinical studies according to the clinical scenarios

Project Number: **FP6-2005-IST-026996**

Deliverable ID: **D 12.1**

Deliverable Name: **Definition of the ACGT clinical studies according to the clinical scenarios**

Date: **November 20, 2006**

COVER AND CONTROL PAGE OF DOCUMENT	
Project Acronym:	ACGT
Project Full Name:	Advancing Clinico-Genomic Clinical Trials on Cancer: Open Grid Services for improving Medical Knowledge Discovery
Document id:	D 12.1
Document name:	Definition of the ACGT clinical studies according to the clinical scenarios
Document type (PU, INT, RE)	PU
Version:	**FINAL**
Date:	20.11. 2006
Authors: Organisation: Address: E-mail:	Dimitris Kafetzopoulos FORTH Science and Technology Parc of Crete, Heraklion, Greece kafetzo@imbb.forth.gr

Document type PU = public, INT = internal, RE = restricted

ABSTRACT

This document presents ACGT deliverable **D12.1**: *Definition of the ACGT clinical studies according to the clinical scenarios*. It unfolds into **four**- (4) chapters. The first chapter (**I**), presents the overall ACGT clinical research objectives and targets. The next three chapters, **II**, **III**, and **IV**, cover the targeted clinico-genomic trials (as specified in the official ACGT DoW document).

- **Breast Cancer.** Carcinoma of the breast remains the most prevalent cancer diagnosed in women in the world. Although breast cancer mortality has declined in the last two decades, breast cancer continues to represent a major threat to the lives and productivity of women. The number of effective treatments for breast cancer is on the rise; however, the benefit from specific treatments to individual patients and the adverse events they experience vary considerably. Efficacy and safety of anticancer therapies may depend on tumor, treatment, and host characteristics. Small variants in the germline DNA sequence (genotype) may lead to different expression of the encoded protein or to the expression of altered protein, and thus to a different health outcome (phenotype). Considerable effort over the last few years has gone into elucidating the genetics, biology, pathology, and clinical outcome of breast cancer using high-throughput gene expression profiling methods. The recent completion of the human genome project and advances in high throughput DNA sequencing and proteomic technologies may contribute to the understanding of interindividual variability in health outcomes. Based on microarrays technology, investigation of gene expression profiles in hereditary breast cancers have illustrated that tumors derived from individuals with *BRCA1* mutations can be distinguished from those with *BRCA2* mutations based on gene expression profiles. Although *BRCA1* and *BRCA2* were initially proposed to be responsible for the majority of inherited breast cancer, more recent population-based studies suggest that they account for a far smaller portion of familial breast cancer, with considerable variation between different populations. The ACGT trial on breast-cancer will investigate pre-operative chemotherapy treatment and responses in order to identify indicative individualised patients' profiles. The whole effort will rely, and anhance, the TOP-trial on breast-cancer.
- **Paediatric Nephroblastoma - Wilms Tumor.** Wilms tumor is the most common malignant renal tumor in children. More than 25 years after introducing preoperative chemotherapy for Wilms´ tumor, the benefits of this approach are well known, resulting in easier operations, with significantly fewer tumor ruptures during surgery, and a favorable stage distribution. Acute toxicity and late effects are minimized without jeopardizing disease-free and overall survival. Clinical trials for Wilms´ tumor should continue to seek risk factors for further stratifying and individualizing treatment. This will improve the cure rates for high risk patients by intensifying therapy and the quality of life for children with more favorable prognosis by lowering therapy to a minimum that is required. Besides the excellent prognosis of children with Wilms tumor there is a well known risk of unnecessarily administered chemotherapy by treating children preoperatively without histologically proven diagnosis. This risk can be abolished by finding a specific marker

for Wilms tumor in serum, which is lacking today. Immunogenic (i.e., provoking an immune response when introduced into the body) tumor-associated antigens (antigens that are presented by tumor cells and normal cells, as opposed to tumor-specific antigens, which are antigens specific to tumor cells) have been reported for a variety of malignant tumors including brain tumors, prostate, lung and colon cancer. The purpose of this ACGT trial is to find such a marker by searching for a pathognomonic antigen pattern in patients with Wilms tumor. Serum from a specific patient will be tested against newly identified Wilms tumor antigens. As a result in each patient there will be a specific pattern of antigens found. This pattern will be correlated to the histological subtype of the tumor, the gene expression profiling of the tumor, the response to chemotherapy and the outcome of the patient.

- ***In sillico* oncology.** A third action of the clinical trials process (in silico oncology trial) concerns the validation, adaptation and optimization of an advanced computational system, the "Oncosimulator", able to simulate within defined limits of reliability tumor growth and tumor and (to a lesser extent) normal tissue response to therapeutic schedules. The special cases of nephroblastoma and breast cancer will be addressed. The simulation models will be based on the essentially "top-down" modeling approach developed by the In Silico Oncolog Group, ICCS. The in silico oncology trial will be based on the two other clinical trials (nephroblastoma SIOP 2001/GPOH and breast cancer TOP trial) following their considerable enhancement in terms of data collection.

KEYWORD LIST: *Breast cancer, molecular signature,, nephroblastoma, preoperative chemotherapy, antigen pattern, in silico oncology, tumour growth, simulation, clinical trial*

MODIFICATION CONTROL			
Version	Date	Status	Author
0.1	23.2.2006	Draft	Christine Desmedt, Christos Sotiriou
0.2	19.5.2006	Draft	Christine Desmedt
0.3	17.7.2006	Draft	Norbert Graf, Alexander Hoppe
0.4	30.7.2006	Draft	George Stamatakos, Norbert Graf
0.5	6.8.2006	Draft	Maria Klapa
1.0	21.8.2006	Draft	D. Kafetzopoulos
1.6	12.11.2006	Draft	D. Kafetzopoulos
2.0	20.11.2006	**Final**	D. Kafetzopoulos

List of Contributors

- **Dimitris Kafetzopoulos**, Institute of Molecular Biology & Biotechnology, FORTH
- **Maria Klapa**, Institute of Molecular Biology & Biotechnology, FORTH
- **Christos Sotiriou**, Medical Oncology Department, Jules Bordet Institute
- **Christine Desmedt**, Medical Oncology Department, Jules Bordet Institute
- **Norbert Graf**, Department of Paediatric Haematology and Oncology, University Hospital of the Saarland
- **Alexander Hoppe**, Department of Paediatric Haematology and Oncology, University Hospital of the Saarland
- **George Stamatakos**, Institute of Communication and Computer Systems, School of Electrical and Computer Engineering, National Technical University of Athens (NTUA)
- **Francesca Buffa,** Gray Cancer Institute**,** Human Cancer Biology & Informatics
- **Pier Giuseppe Pelicci,** IEO – European Institute of Oncology, Molecular Mechanisms of Cancer and Aging in Mammals

Contents

Executive Summary

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- **Paediatric Nephroblastoma - Wilms Tumor.** Wilms tumor is the most common malignant renal tumor in children. More than 25 years after introducing preoperative chemotherapy for Wilms´ tumor, the benefits of this approach are well known, resulting in easier operations, with significantly fewer tumor ruptures during surgery, and a favorable stage distribution. Acute toxicity and late effects are minimized without jeopardizing disease-free and overall survival. Clinical trials for Wilms´ tumor should continue to seek risk factors for further stratifying and individualizing treatment. This will improve the cure rates for high risk patients by intensifying therapy and the quality of life for children with more favorable prognosis by lowering therapy to a minimum that is required. Besides the excellent prognosis of children with Wilms tumor there is a well known risk of unnecessarily administered chemotherapy by treating children preoperatively without histologically proven diagnosis. This risk can be abolished by finding a specific marker for Wilms tumor in serum, which is lacking today. Immunogenic (i.e., provoking an immune response when introduced into the body) tumor-associated antigens (antigens that are presented by tumor cells and normal cells, as opposed to tumor-specific antigens, which are antigens specific to tumor cells) have been reported for a variety of malignant tumors including brain tumors, prostate, lung and colon cancer. The purpose of this ACGT trial is to find such a marker by searching for a pathognomonic antigen pattern in patients with Wilms tumor. Serum from a specific patient will be tested against newly identified Wilms tumor antigens. As a result in each patient there will be a specific pattern of antigens found. This pattern will be correlated to the histological subtype of the tumor, the gene expression profiling of the tumor, the response to chemotherapy and the outcome of the patient.

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I. Clinical Research in ACGT

I.1 Advanced Clinical Research in the Post–Genomic Era

The seminal publication of the human genome has heralded a new era in medicine. The sequence of more than 98% of the three billion nucleotides of the human genome3 has illustrated that an estimated 34,000 genes are present, and, counting splice variants, the number of functionally distinct proteins is likely to exceed 100,000. We now have access to the "kit of parts" of human biology; however, this is only the first stage in understanding how humans develop and function and what happens to our bodies when disease strikes. The sequence of human genes provides first-order understanding of their corresponding protein products. The genome sequence does not, however, allow an immediate understanding of the physiologic circumstances under which these proteins are produced and function in the cell.

As new analytical strategies undergo testing and validation in clinical research settings, they increasingly acquire the potential to drive significant advances in oncology. DNA-based assays are rapidly evolving as a result of an expansion in the repertoire of nucleic acid amplification methods and the availability of accurate gene annotation and sequences. The development of low-cost instruments and probes for real-time fluorescence monitoring of DNA amplification reactions is facilitating the application of these new methods. Improved methodology for whole genome amplification has removed former technical hurdles for the analysis of very small tissue samples. Additionally, the introduction of microarray formats for the analysis of DNA as well as proteins has stimulated the adoption of comprehensive molecular analysis as a valid research paradigm in oncology. The cancer investigator need no longer limit analysis to a few genes of interest but is readily able to gather information about thousands of molecular targets in parallel.

I.1.1 Molecular Profiling in Cancer

Genes are regulated at multiple levels (transcriptional, posttranscriptional, translational, and posttranslational) to produce the delicate balance of a fully functional organism. Nevertheless, gene-specific transcription is one of the major gene regulatory steps in the cell and is influenced by cell type and differentiation stage, as well as external stimuli. Although it may not be currently understood how all of the circuits regulating gene expression function, defects in these circuits that characterize biologically distinct disease states can be identified by ascertaining the amount of each transcript that is being produced.

Using DNA microarrays to simultaneously evaluate the level of transcription of thousands of genes ("expression profiling") is one means to visualize cellular transcriptional circuitry and has tremendous potential for advancing the understanding of human cancers. The vast majority of human cancers display marked aneuploidy, multiple genetic alterations, and/or genetic instability; this complexity most likely attributes to the diversity in clinical outcome of histopathologically similar cancers. The downstream effects of these complex changes, however, have proven difficult to investigate with traditional gene-by-gene methods. Because many of these genetic changes alter the transcription of specific groups of genes, expression microarrays are an attractive platform for characterizing the changes associated with specific cancers as well as facilitating a global, comprehensive view of the biologic changes attributable to these alterations.

A major focus in cancer research today, now greatly aided by the use of microarrays, lies in identifying genetic markers that can be used for precise diagnosis and as targets for novel therapies and in translating these findings into the clinic. Microarrays can be used to subclassify tumors into homogeneous entities based on gene expression profiles; such subgroups of specific cancers may represent distinct disease states that respond differently to currently used therapies. In addition, genome-wide expression data can help in further characterizing the biology of these "new" subgroups. Finally, microarray experiments can aid in the search for new therapeutic targets and in the identification of novel diagnostic markers. Significant strides have been made toward these goals for several types of malignancies for which large numbers of frozen samples suitable for RNA analysis had been previously assembled. As a result, the use of DNA microarrays is now nearing implementation into clinical practice for several such malignancies, as are promising new therapies whose target was identified through expression profiling.

I.2 Microarray Technology

I.2.1 Microarray Experiment Overview

Although various microarray platforms [complementary DNA (cDNA) and oligonucleotide] may use diverse manufacturing, labeling, and analysis methods, the general principle of a microarray experiment remains the same. For each sample to be analyzed, RNA is first extracted. The messenger RNA (mRNA) is subsequently copied in an enzymatic reaction using a reverse transcriptase and labeled nucleotides (usually fluorescent), thereby generating labeled cDNA. This labeled cDNA is subsequently applied to the surface of a microarray, which contains thousands of cDNA or oligonucleotide probes, each derived from the coding sequence of individual human genes and located at a unique location on the array surface. As the cDNA is incubated on the microarray, labeled cDNA molecules hybridize to the microarray spot representing their respective genes. After hybridization, the array is washed and scanned using a fluorescence microscope, and the degree of fluorescence at each microarray spot is quantitated. Thus, a disparity in the abundance of any specific gene transcript between two samples is reflected by differences in the fluorescent intensity at the spot representing that gene on the microarray.

I.2.2 Microarray Data Analysis: Classification of Human Tumors

Although new array analysis methods to classify tumors are continuously being developed and are becoming increasingly sophisticated and computationally intensive,4 two approaches, in general, are commonly used to classify cancers using gene expression profiling data. Unsupervised analyses typically use pattern-recognition algorithms to define groups of samples that have similar global patterns of gene expression. Likewise, such analysis also identifies genes whose expression pattern is similar across a set of samples. Unsupervised analyses minimize a priori assumptions about the data and thus identify structure in array data without regard to known clinical parameters. Thus, such analysis is useful for distinguishing subgroups of cancer that differ from each other in the expression of large numbers of genes, presumably unique biologic entities. Indeed, unsupervised analysis of microarray data has successfully separated subgroups of cancer that are known to differ significantly in terms of biology as well as clinical outcome, for example, estrogen receptor–positive (ER+) and –negative (ER–) breast cancers. Despite the fact that the genes selected for this analysis were not chosen in advance on the basis of correlation with any clinical parameter, tumors with a similar phenotypic characteristic (ER status) were largely grouped together on the basis of global patterns of gene expression, suggesting that these samples are biologically

similar. Likewise, genes with similar patterns of expression, including those that distinguish ER+ and ER– tumors, are grouped, suggesting that these genes may be commonly regulated in an ER-dependent manner.

Although unsupervised analyses are effective at classifying tumors that have similar expression patterns for a large number of genes, such analyses are far less effective at identifying differences in the expression of small numbers of genes that nonetheless correlate with clinical parameters, including response to therapy. Such genes may be useful as markers for the development of differentiating tests that refine our ability to classify tumors and predict response to therapy beyond that achievable using current clinical data or array-based unsupervised classification methods, or both. Identifying these relationships often requires supervised analysis, in which statistical algorithms are used to identify genes whose expression is significantly correlated with a specific clinical parameter such as outcome. The power of these genes may be subsequently validated on an independent set of tumors by clinically classifying these samples based only on the expression levels of these preselected genes alone.

Because supervised analyses use only those genes that best correlate with specific clinical parameters, they frequently classify tumors according to those parameters better than do unsupervised methods. In the case of breast cancers, for example, ER+ and ER– tumors are better classified when only the genes that best distinguish these two subgroups are considered.

I.2.3 Toward Translation of Microarray Research to the Clinic

Retrospective expression profiling of human cancers has in recent years led to a greater understanding of the heterogeneity underlying numerous types of malignancies, particularly those that are readily resectable and for which frozen biopsies were frequently archived in the past (e.g., leukemias). Several malignancies, in particular, have been quite well studied, leading to improvements in the ability to subclassify clinically heterogeneous cancers and predict patient outcome and, in some cases, to the identification of novel therapies. Presented here is a synopsis of the progress made through microarray expression profiling for three well-studied cancers: ALL; diffuse large B-cell lymphoma (DLBCL); and breast cancer. The advancements made for these cancers are slowly being translated into clinical practice, and for breast cancer in particular, microarray-based diagnostics are now being used to identify good-prognosis patients who do not need to receive adjuvant therapy after surgery. Finally, insights into the process of metastasis, revealed by comparative analyses of large microarray-based studies in numerous malignancies, including lung, breast, prostate, and brain cancer, are discussed. Such analyses are beginning to lead to a better understanding of common mechanisms that influence the progression of multiple types of tumors. The work presented here demonstrates the potential of genomic expression analysis to revolutionize the diagnosis and treatment of human cancers.

I.2.4 Microarrays, Tumor Aggressiveness, and Metastasis

In addition to leading to a better understanding of the molecular events involved in tumorigenesis of specific cancers, microarray studies are beginning to reveal clues to genetic influences that affect tumor progression across a broad spectrum of cancers. To date, several microarray studies have attempted to correlate gene expression with aggressive tumor phenotypes. Expression profiling studies have thus identified gene expression signatures associated with tumor cell aggressiveness in melanoma. Among the genes most preferentially expressed by highly invasive melanoma cells, *WNT5A*

expression and signaling through the *WNT5A* receptor (*FZD5*) have been shown to have a functional role in mediating the invasive phenotype.

Likewise, expression profiling studies have identified expression signatures associated with the eventual development of metastases in primary breast tumors, as well as signatures correlating with the presence of metastases at the time of diagnosis in childhood medulloblastoma. Most recently, expression profiled a collection of primary lung adenocarcinomas as well as lung adenocarcinoma metastases and identified a 17-gene molecular signature preferentially expressed by metastases. Strikingly, a small proportion of primary tumors expressed this metastasis signature, leading the authors to hypothesize that these specific primary tumors may have an inherent "metastatic program." In support of this hypothesis, individuals with primary tumors that expressed this metastasis signature had a much poorer outcome than those with a nonmetastatic signature. To determine whether this 17-gene signature held any relevance for other types of cancer, the authors subsequently applied this metastasis predictor to previously published microarray data sets from breast cancer, prostate cancer, medulloblastoma, and lymphoma. Intriguingly, for the three solid tumor types, stratification of patients based on the expression of these genes resulted in groups that differed significantly in clinical outcome, with the metastasis signature associated with poor survival.

The observation of "metastasis signatures" in primary tumors has led many to suggest that the prevailing model of metastasis, in which only rare cells in a tumor acquire the metastatic phenotype, may need to be reconsidered. Because of limitations in the sensitivity of DNA microarrays, the presence of a metastasis signature in a primary tumor suggests that the proportion of cells in the tumor that have acquired metastatic characteristics must be large; metastasis-specific changes in expression that occur in only a very rare population of cells would not likely be detectable. That a large proportion of cells in a tumor express metastasis marker genes, then, suggests that at least some of the changes that potentiate metastasis may be early changes that also promote noninvasive growth.

This hypothesis is supported by the results of a genetic screen for metastatic potential in *Drosophila*, in which the oncogenic background of a tumor was shown to contribute toward metastasis formation. Although inactivation of specific cell polarity genes promotes the formation of metastases in eye disc tumors initiated by mutations to the *Ras* oncogene, inactivation of these same cell polarity genes did not influence metastasis development in the context of tumor-initiating mutations to the *lats* tumor suppressor gene. It has alternatively been suggested that a patient's inherited genetic background, reflected within the gene expression of their tumor, may contribute to metastatic potential; the genetic background of mice appears to influence the frequency of metastasis in a transgene-induced mouse tumor model. Still, it is a point of contention whether any of these data are truly inconsistent with the prevalent model of metastasis; it has been suggested that although many of the cells may acquire *some* of the characteristics that allow for successful metastasis early during progression, it is still the rare cell that acquires *all* of these characteristics. Nonetheless, these data highlight the fact that early genetic events critical for early tumor progression may also influence the later potential of a tumor to metastasize.

I.2.5 Large-Scale Clinical Trials to Validate Predictors

Larger clinical trials validating the accuracy of expression profiling as a prognostic tool are clearly warranted. Even in studies of large patient cohorts, some biologically distinct groups of patients may be represented in only small numbers. Robust classifiers that work well for predicting outcome in well-represented patient populations may not, in fact,

work well in underrepresented groups. For example, in the breast cancer classifier developed by van't Veer et al., the prognostic profile developed was associated with, among other parameters, ER status. Consistent with the key role of ER in breast cancer outcome, the vast majority of tumors examined within the good-prognosis group were ER+. Thus, it is not altogether clear how well this predictor will work with a larger cohort of ER– samples. Because the etiology of cancer is complex and in many cases influenced by environmental exposures and genetic background, future studies aimed at validating outcome predictors for various cancers will also need to address accuracy across diverse ethnic or geographic populations, or both. Clearly, prospective microarray studies based on larger patient cohorts representing the whole spectrum of any given cancer are needed to refine prognostic models that are truly ready for routine clinical use.

I.3 New Therapies for High-Risk Patients

For many cancers, the ability to better predict outcome may immediately improve patient survival; high-risk patients may receive intensified therapy at the time of diagnosis, whereas groups of low-risk patients who do not require such therapy to be cured may be spared treatment-related risks. However, for many diseases that lack effective alternative therapies for high-risk subgroups, such prognostics are of little immediate value to the patient. Identification of therapeutic targets for the treatment of these subgroups is of critical importance. In the short term, many of the genes that define high-risk subgroups may prove to be viable targets for drugs that are currently in development or already in clinical trials for other types of cancer.

Still, the development of new therapies based on microarray data has been a slow process, on the whole, for several reasons. Effective therapies against many potentially attractive therapeutic targets identified via microarray experiments, for example, DNA-binding transcription factors, are lacking. The development of agents that effectively target these molecular markers is required for the potential of these therapeutic targets to be realized. Still other genes whose expression defines a high-risk cancer subgroup may not themselves be attractive therapeutic targets at all, as altering the expression or function of these genes alone may not dramatically impact the tumorigenic process. The altered expression of such genes may instead be a downstream readout of more important upstream oncogenic events critical to tumorigenesis. Numerous bioinformatic tools that may recognize gene expression patterns attributable to such "hidden" oncogenic events are currently in development. These tools, however, will be much improved in the future with the continued generation of basic research data characterizing cellular transcriptional responses to various oncogenic stimuli, particularly in controlled *in vitro* systems. Finally, when a new druggable therapeutic target is identified, a tremendous amount of validation work may be required, in *in vitro* and in animal models, before any such novel therapies are moved into human clinical trials. Thus, the larger impact of microarray technology on clinical practice may be felt more in the long term.

I.3.1 Integration of Data from Other Genomic Technologies

A number of other relatively new genomic technologies are emerging that stand poised to contribute significantly to the goal of improving cancer diagnosis and survival. Importantly, these methods may provide unique prognostic and biologic insight into the pathogenesis of human cancers that cannot be derived from expression microarray data. Specifically, somatic changes associated with specific cancers may dramatically influence tumor phenotype. Array-based CGH for identifying DNA copy number changes in individual genes may provide a useful tool for cancer classification and prediction of

response to therapy. Array CGH can be used in conjunction with expression arrays to quantitate the impact of genomic changes on gene expression. Likewise, mutation detection methods have already proved to be useful, revealing common mechanisms of tumor progression. In some cases, such as activating *KIT* mutations in gastrointestinal stromal tumors, the discovery of such mutations has led to the implementation of new therapies.

Inherited differences in an individual's genetic background may also dramatically influence tumor growth and metastasis, as well as response to therapy (drug metabolism by the body). Such differences may not be substantially reflected in tumor gene expression levels but could nonetheless have a dramatic effect on treatment outcome. Thus, large-scale efforts to identify single-nucleotide polymorphisms that are responsible for, or associated with, such differences will doubtless be important; such information would greatly complement gene expression–based diagnostics.

Lastly, proteomic methods have tremendous potential to detect differences between tumors in regard to the complement of proteins expressed by a cell, as well as their respective states of posttranslational modification. Protein spectra of individual tumors can be used in a similar manner to microarrays for the classification of tumors and identification of molecular therapeutic targets. Furthermore, the tremendous sensitivity associated with some proteomic methods also makes them ideal for the identification of serum markers for the detection of residual disease, circulating metastases, or the early detection of cancers.

I.4 Concluding Remarks

As a result of the power of DNA microarray analysis, the future will likely continue to bring substantial changes to the molecular and pathologic classification of tumors. Although progress has certainly been made in the study of many cancers, gene expression in numerous malignancies remains minimally studied on a genome-wide level. Tissue access remains perhaps the largest hurdle for the study of most of these cancers. For many cancers, tumor tissue is not easily resected or biopsied, precluding the assembly of large sample sets for expression profiling. For other tumors, biopsies are small, and many institutions or researchers are understandably reluctant to part with such precious tissue, some or all of which may be consumed by a single microarray experiment. Ongoing advances in RNA amplification and microarray labeling methods promise to greatly reduce the amount of tissue needed for reliable genome-scale expression analysis down to perhaps 100 cells. Last, although many individual clinicians and clinical institutions have begun to archive frozen specimens regularly with the goal of amassing sample sets large enough for genomic analysis, a single institution may not amass a cohort of specimens large enough to sample a genetically heterogeneous tumor type adequately, even after years of prospective collection. Efforts are under way to address this concern, including a National Biospecimen Network. Although no consensus on a specimen acquisition platform has yet been reached, the National Biospecimen Network would serve to collect well-annotated tissue specimens and distribute these resources to the research community.

The pooling of resources from multiple clinical institutions is clearly required to accelerate the discovery process for cancer research. The pace of tissue accrual would be quickened by the regular archiving of frozen tissues by all clinical institutions, not just those with genomic research programs. Clinicians at these institutions could be benefited by entering into collaborations with genomics research groups that are actively amassing large, multi-institutional sample sets for microarray study; indeed, many of the large

sample sets published to date represent such multi-institutional collaborations. Alternatively, such institutions could also benefit the research community as a whole by submission of well-annotated, frozen specimens to cooperative tissue banks that distribute these samples to the research community. Finally, such samples could also be submitted to large molecular profiling organizations that expression profile cancers and publicly disseminate expression profiling data.

Continuing efforts to study the gene expression of cancers on a genome-wide scale will ultimately result in advances in patient treatment. Past microarray studies have demonstrated that gene expression profiles are capable of confirming the major histopathologic distinctions described in cancer, as well as further defining phenotypically indistinguishable tumor subsets as biologically distinguishable entities. These novel subsets sometimes harbor subtle molecular changes that appear to have a significant impact on prognosis and response to therapy. Indeed, the variability in clinical behavior reflects the heterogeneity of tumors, and it appears likely that the introduction of transcriptional profiling technologies into the clinical setting will make possible the individualization of treatments and greatly improve the efficacy of anticancer therapy. Moreover, large-scale expression analysis will likely become increasingly useful in the search for novel therapeutic targets, as well as in the establishment of new prognostic markers for disease. The initial studies in this exciting field of genetic cancer research await confirmation in independent analyses, but it appears likely that molecular profiling using disease-specific microarrays will regularly be incorporated into clinical practice within the not-too-distant future.

II. The ACGT Nephroblastoma Research

II.1 Nephroblastoma Historical Bacground

Cure rates of nephroblastoma have been increasing all over the world. This is largely due to good cooperation between multidisciplinary teams in several parts of the world. For Europe and some countries outside the continent the SIOP studies gave the key for treatment strategies. For North America it was the National Wilms' Tumor Study Group (NWTSG), but also other national groups like the United Kingdom Children's Cancer Study Group (UKCCSG) and the Brazilian Wilms' Tumor Study Group have contributed to the now standing results. A schematic survey of the most important studies is given.

II.1.1 International Society of Paediatric Oncology (SIOP)

II.1.1.1 SIOP 1

This study was open for registration from September 1971 until October. 1974. The number of registered patients was 398. In this study there were two randomized questions. Outline of the study and the answers are given here below.

	Randomization	Outcome
Diagnosis	R → RT - S – RT / S - RT	4% ruptures (p=0.001) 31% stage 1 32% ruptures 14% stage 1
Surgery	R → Act-D 1 course / Act-D 6 courses	no difference in DFS/S

In conclusion: Pretreatment reduces the number of ruptures and favours the stage distribution after surgery. There is no evidence that Act-D after surgery contributes in this study to a better disease free survival and/or survival (93).

II.1.1.2 SIOP 2

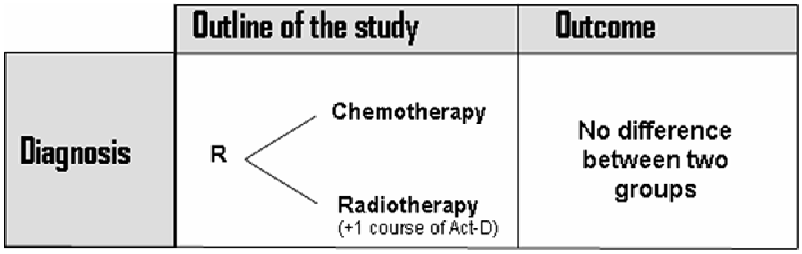
This study was open for registration from October 1974 to December 1976 and 138 patients were included in the study period. It was a non-randomized study to confirm the findings of SIOP 1.

	Outline of the study	Outcome
VCR added to Act-D postop	R → 9 months / 15 months	DFS/S equal
Ruptures	R → Preop. RT / Primary surgery (Various reasons: e.g. small tumors)	5% (p = 0.0025) 20%

In conclusion: It is not necessary to give a two drug combination for more than 9 months postoperatively. Beware of the temptation to operate on small tumours.

II.1.1.3 SIOP 5

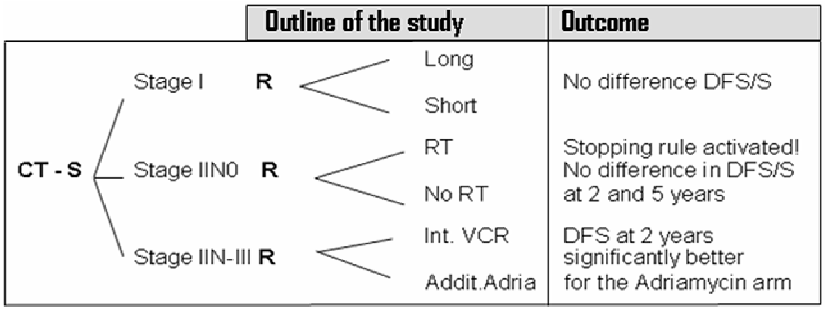
This study was open for registration from January 1977 until July 1979. The number of registered patients was 433. The number of registered patients was 433. Outline of the study is given here below:



In conclusion: Chemotherapy is comparable to radiotherapy in efficacy of preparing the tumor for surgery. Due to less late effects it is preferable to use drugs instead of radiation (94).

II.1.1.4 SIOP 6

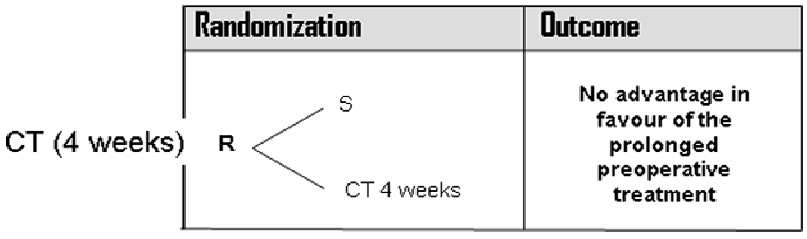
This study was open for registration from July 1980 to October 1987 (for stage IIN0 until April 1986). The number of registered patients was 1095. There were three randomized questions. Outline of the study and answers are given here below:



In conclusion: After pretreatment and surgery only a short postoperative treatment is necessary in stage I patients. In stage IIN0 there are relapses but not only in the tumourbed. So intensification of chemotherapy seems to be the way to go. In the more advanced stages Adria proved to be an effective supplement to VCR and Act-D (150).

II.1.1.5 SIOP 9

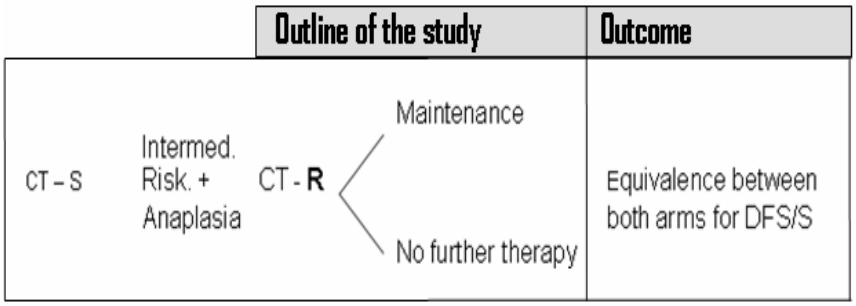
This study was open for registration from November 1987 to November 1991. The number of registered patients was 852. Outline of the trial is given here below:



In conclusion: Eight weeks does not seem to improve stage distribution. Even if the tumours in general do shrink a little bit more after another 4 weeks of chemotherapy, this does not mean a significant improvement (153).

II.1.1.6 SIOP 93-01

This study was open for registration from July 1993 until August 1999 and the number of registered patients was 1104 (December 1998). Outline of the trial with a randomization in the stage I group with intermediate risk or anaplasia, is given here below:

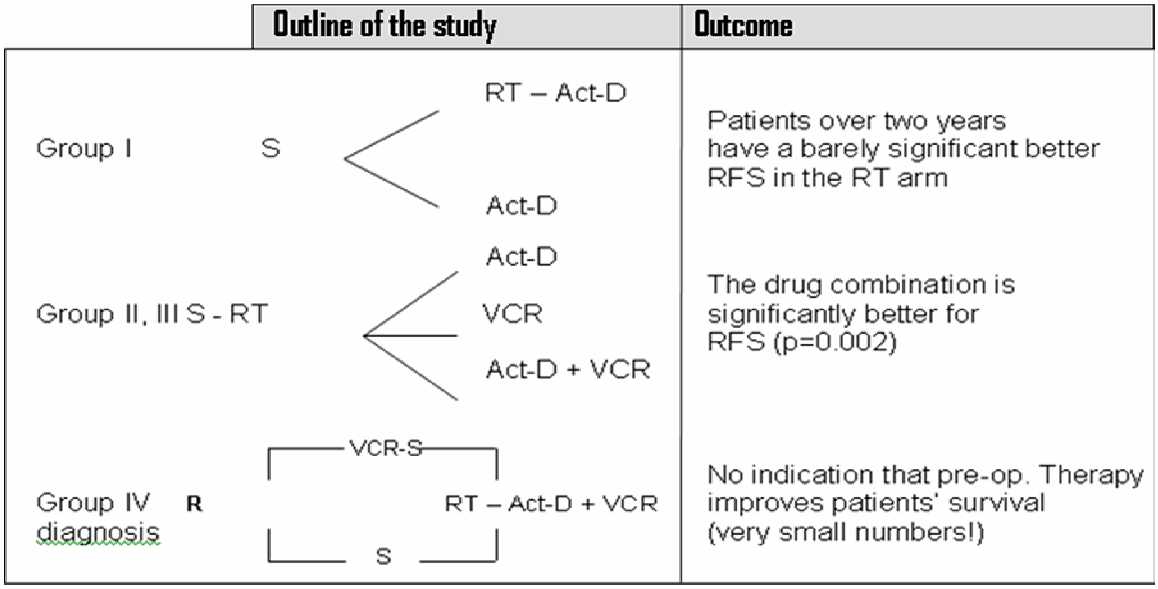


In conclusion: Another reduction in postoperative therapy in this group of patients is feasable. The results are published in Lancet (35). Further results are the poorer outcome of patients with blastemal predominant histology. These patients will be treated as high risk patients in the ongoing SIOP 2001 trial.

II.1.2 National Wilms Tumor Study

II.1.2.1 NWTS 1

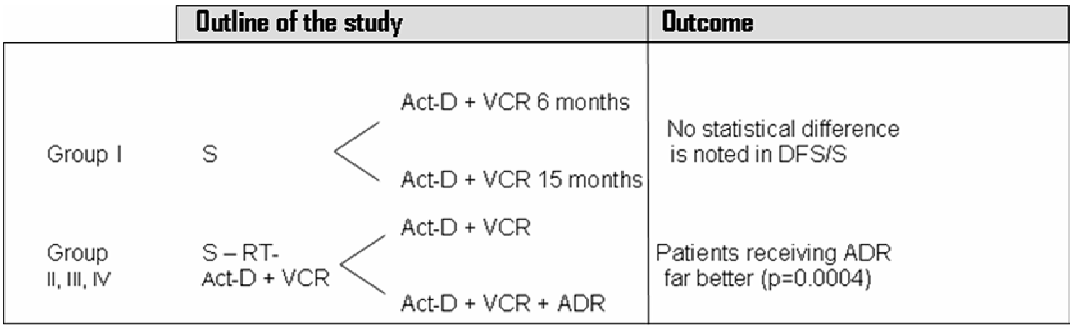
This study was open for registration from October 1969 until December 1973. The number of registered patients was 606.



In conclusion: Radiotherapy is effective for selected patients. The combination of VCR and Act-D is better than either drug alone. No evidence for a role of preoperative VCR but too small numbers to be conclusive (29).

II.1.2.2 NWTS 2

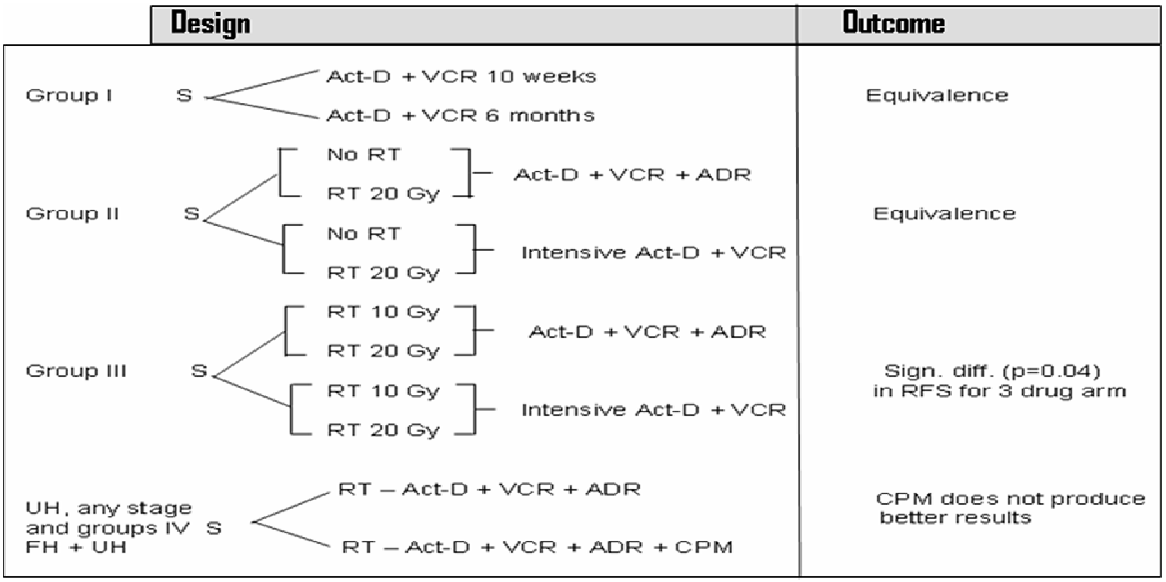
The study was open for registration from January 1975 until July 1978 (group I from October 1974). The number of registered patients was 755.



In conclusion: There is no indication that a long postoperative treatment is of any use. Adriamycine is an effective drug in Wilms' tumour (31).

II.1.2.3 NWTS 3

This study was open for registration from May 1979 to May 1985 (November 1985 for stage I, FH). The total number of registered patients was 2496.



In conclusion: Again it is shown that in low-stage tumours postoperative chemotherapy can be short. Radiotherapy, if necessary, does not have to be of high dose. Adriamycin adds to the effect of the well known two drug combination and cyclo is so far not a promising drug (32).

II.1.2.4 NWTS 4

The study was open for registration from August 1986 until September 1994 and 905 previously untreated children were randomized. Either for duration of treatment and/or for single dose versus divided dose of drug administration. This study was to evaluate the efficacy, toxicity and costs of the administration of different regimens for the treatment of WT.

In conclusion: The short administration for the treatment of children with WT is no less effective than the long one and can be administered at a substantially lower total treatment cost (54).

II.1.2.5 NWTS 5

Between August 1995 and June 2002, 2.021 previously untreated children with FH or anaplastic Wilms tumor, clear-cell sarcoma of the kidney (CCSK) or malignant rhabdoid tumor of the kidney (RTK), were treated with stage- and histology-specific therapy. Their tumors were assayed for LOH for polymorphic DNA markers on chromosomes 1p and 16q.

In conclusion: Tumor-specific LOH for both chromosomes 1p and 16q identifies a subset of FH Wilms tumor patients who have a significantly increased risk of relapse and death. LOH for these chromosomal regions can now be used as an independent prognostic factor together with disease stage to target intensity of treatment to risk of treatment failure (68).

II.1.3 United Kingdom Wilms Tunour Studies

II.1.3.1 UKW1

This study ran from 1980-86 and recruited 384 patients (~80% of all UK cases of Wilms tumour). Immediate nephrectomy was recommended for all non-metastatic tumours. Aims were 1) to reduce treatment for low stage, FH disease (omit RT for stage I, omit adriamycin and reduce dose RT for stage II, without impairing survival, and 2) to intensify treatment for stage III and IV and for UH tumours to improve survival.

	Outline of study	Outcome
Stage I (n=104)	single agent VCR x 26 wks, omit RT	89% 6yr EFS, 96% OS. single agent VCR effective
Stage II (n=54)	two drugs VCR/ActD x 26 wks, RT	85% 6yr EFS, 93% OS safe to omit adriamycin
Stage III (n=106)	Sequential VA + ADR x 1 yr, RT	82% 6yr EFS, 83% OS
Stage IV (n=40)	VA + ADR + cyclo x 1yr. lung RT only if no remission at wk 12	50% 6yr EFS, 65% OS

In conclusion: Outcome for FH stage I disease equivalent to NWTS 2 – 4 and avoids use of ActD. Similar outcome to NWTS 2 & 3 for stage II and III tumours suggests pulsed ActD is as effective as fractionated regimen. Inferior results for stage IV disease may be due to small number receiving RT (4/40) compared to NWTS approach (117).

II.1.3.2 UKW2

This study ran from 1986-91 and recruited 448 patients (> 90% of all UK cases of paediatric Wilms tumour). Immediate nephrectomy was recommended for all non-metastatic tumours. Aims were 1) to further reduce treatment for low stage, FH disease by reducing duration of VCR to 10 weeks for stage I and omitting RT for stage II disease, 2) to improve outcome for stage IV, primary 'inoperable' and UH tumours by intensifying chemotherapy with simultaneous administration of ActD and ADR ("intensive AVA").

	Outline of study	Outcome
Stage I (n=136)	VCR x 10 wks	87% 4yr EFS, 94% OS.
Stage II (n=57)	two drugs (VA) x 26 wks, no RT	82% 4yr EFS, 91% OS
Stage III (n=122)	Sequential VA + ADR x 1 yr, RT	82% 4yr EFS, 84% OS
Stage IV (n=60)	intensive AVA x 1yr, lung RT recommended for all	70% 4yr EFS, 75% OS

In conclusion: Excellent outcome for stage I FH maintained with only 10 weekly VCR. Stage II FH requires only 2 drugs (VA) and no RT. Improved stage IV survival but still inferior to NWTS results – may be due to reduced use of RT despite protocol recommendation (only 37/60 received lung RT) (105).

II.2 Rational and objectives for SIOP 2001

II.2.1 Introduction

The Nephroblastoma clinical trial and study SIOP 2001 is a continuation of the philosophy of the former SIOP studies. The basic idea has always been: Collect a lot of reliable data by working together on an international base and answer questions which can be of direct importance for the outcome of the patients.

Specific objectives for SIOP 2001 are:

- To adapt therapy to the known individual risk of the patient, and
  - ♦ increase survival for blastemal predominant tumours after preoperative chemotherapy by intensifying therapy;
  - ♦ minimise acute and late toxicity without jeopardising event free survival and survival by reducing treatment
    - ♦ for patients with focal anaplasia,
    - ♦ for stage I patients with intermediate risk tumours,
    - ♦ and for stage II and III patients with intermediate risk tumours by randomising doxorubicin
- To test the treatment hypothesis that doxorubicin is not necessary in patients with intermediate risk tumours and local stage II or III by a multicentric prospective randomised trial
- To prospectively analyse different histological components of nephroblastoma with a special emphasis on a percentage of blastemal component which might be of prognostic significance
- To reduce the number of drug administrations, hospital visits and thereby costs in the preoperative phase
- To collect material for performing biological studies with specific aims
- SIOP 2001 is based on the results of the previous SIOP trials and studies as well as on the results of the NWTS protocols. The study design and the logistic of the study was made as simple as possible, because of the world wide participation of centres.

II.2.2 Pre-operative treatment

The previous SIOP studies showed the effectiveness of pre-operative chemotherapy:

- by reducing the risk of tumour rupture during surgery
- by inducing a favourable stage distribution with 60 % stage I patients requiring less post-operative therapy
- by selecting "good responders" in stage IV patients and
- by providing the opportunity of partial nephrectomy in an increasing number of patients

Furthermore the use of pre-operative chemotherapy changes the distribution pattern as well as the prognostic value of the different histological subtypes compared to immediately operated tumours (Fig. 1).

This information can be used as a measure for response to pre-operative chemotherapy and helps to better stratify post-operative chemotherapy according to the individual risk of the patient. Besides this the tumour volume after pre-operative chemotherapy is dependent on histology and seems to correlate with prognosis (Fig. 2, Fig 3). So tumour volume might be used as a parameter for response to pre-operative chemotherapy. This hypothesis will be tested in SIOP 2001 prospectively. To have an answer on this question, it is mandatory to measure the tumour volume at diagnosis and after preoperative chemotherapy precisely. The measured tumour volume will be correlated to the specimen weight, to find the best and easiest way to measure "tumour volume".

The use of pre-operative chemotherapy will be continued in SIOP 2001. It will be given to all patients between 6 months and 18 years of age, if imaging studies confirm the diagnosis of nephroblastoma.

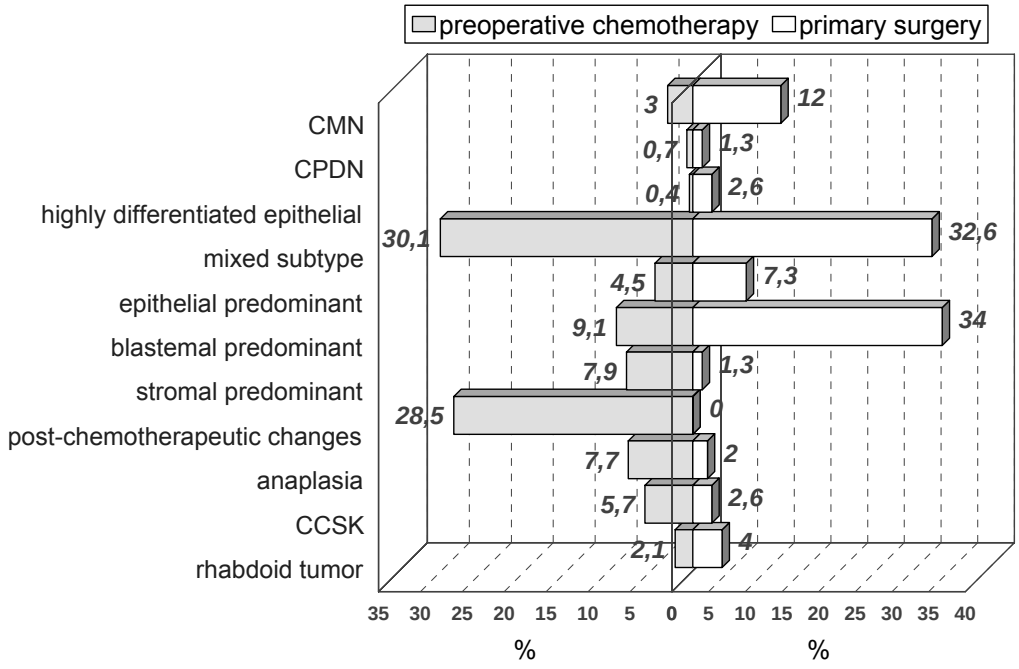


Figure 1. Distribution of histological subtypes in registered patients from the GPOH subgroup of SIOP 9 and SIOP 93-01 according to the initial treatment.

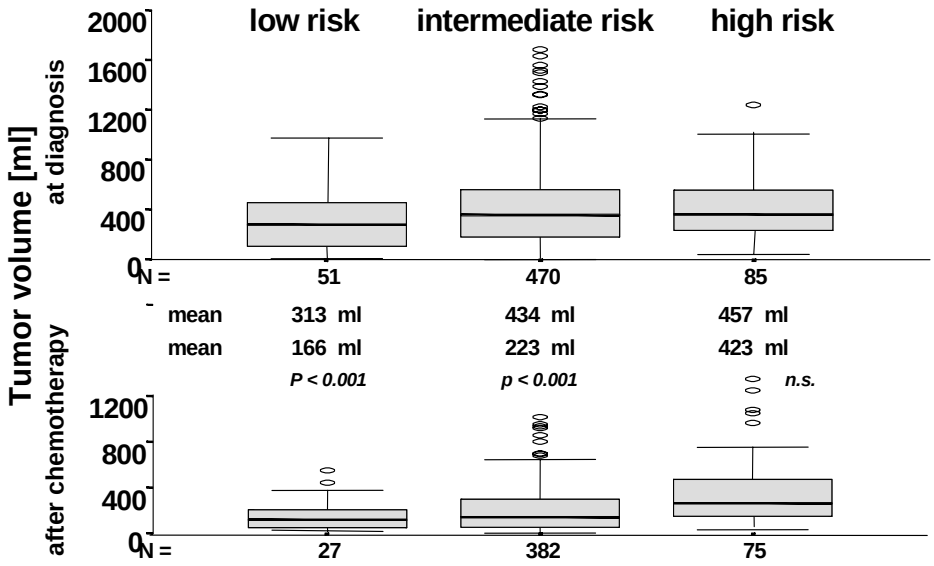


Figure 2. Tumor volume at diagnosis and after preoperative chemotherapy according to histology.

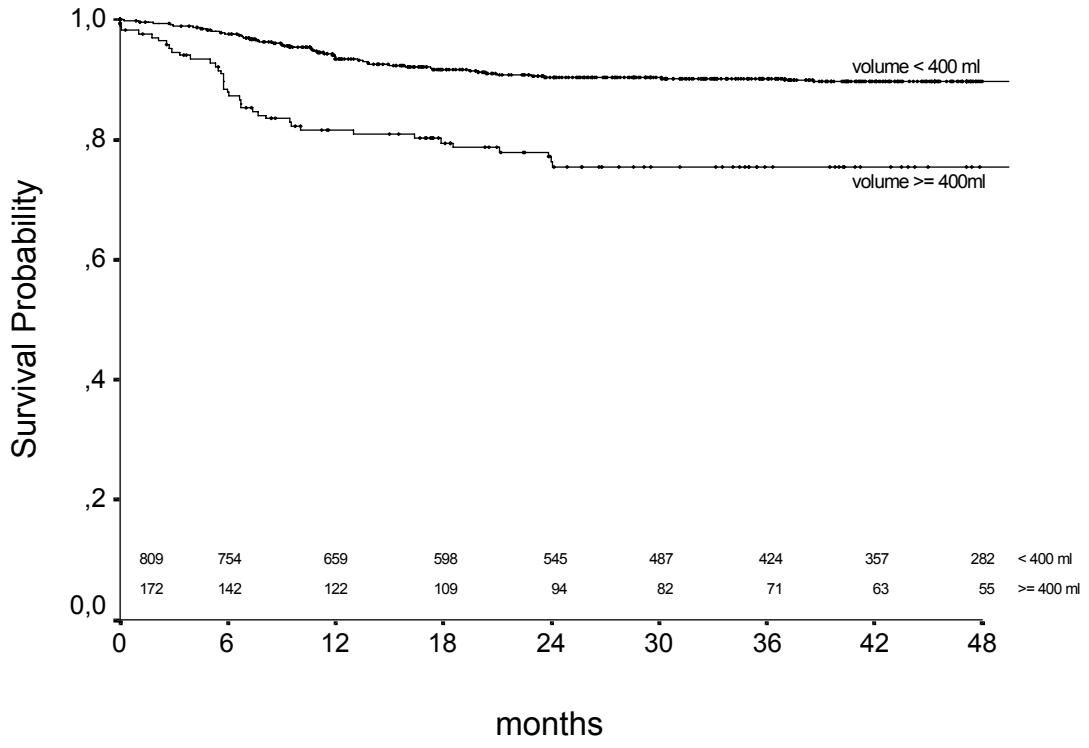


Figure 3. Event free survival according to tumour volume after preoperative chemotherapy.

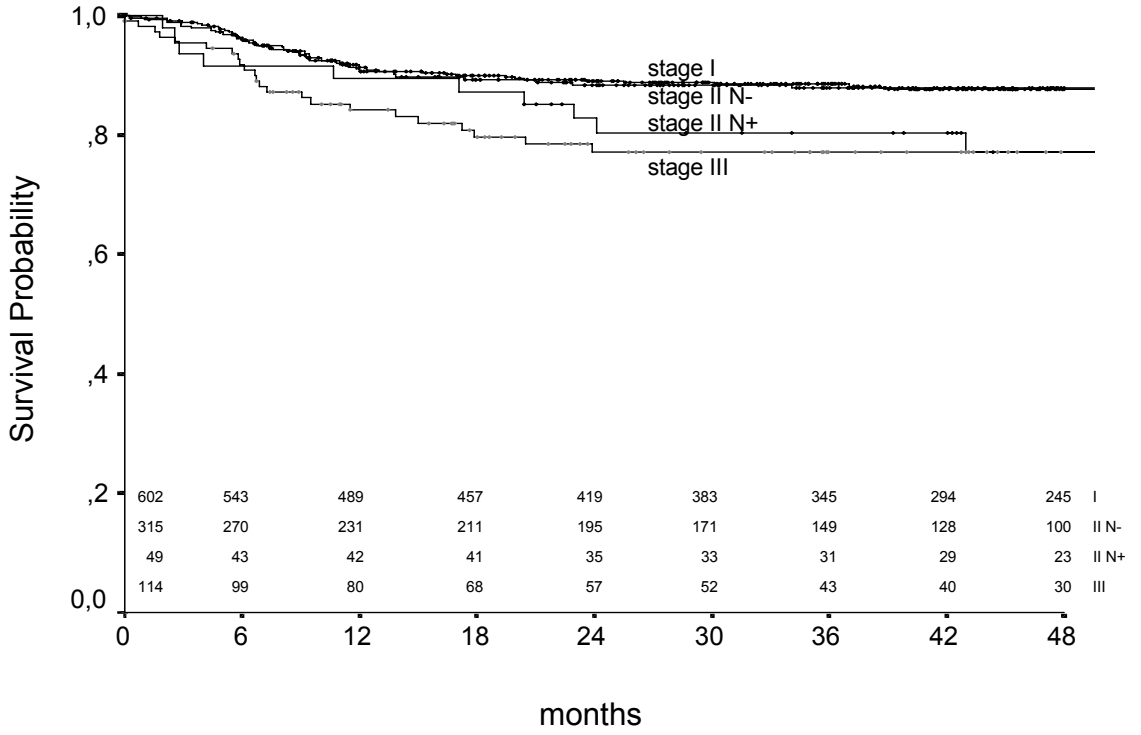


Figure 4. Event free survival by stage done by the panel of pathologists.

II.2.3 Post-operative local stage & histological classification

Regarding SIOP stage and prognosis there is no discrimination between stage I and II N- and between stage III and II N+ (Fig. 4). In order to better compare SIOP results with the results of the NWTS studies a stage II N+ tumour is defined as a stage III tumour in SIOP 2001.

A retrospective analysis of SIOP 93-01 data looking for risk factors showed that the histological classification of the tumour as low, intermediate or high risk is most important.

There have been changes in all three risk groups. In the low risk tumour group there is only one tumour type: completely necrotic nephroblastoma (cystic partially differentiated nephroblastoma and mesoblastic nephroma, when diagnosed on imaging studies, should be treated with surgery only). The intermediate risk tumour group consists of five types including epithelial, stromal, mixed and regressive type and focal anaplasia. Focal anaplasia has a better prognosis than diffuse anaplasia with an event free survival lying within the range of other intermediate risk tumours, so treated accordingly. Finally, in the high risk tumour group there are blastemal type nephroblastoma which showed to have a poorer outcome than any other intermediate risk tumour, and diffuse anaplasia. These tumours will be treated as high risk tumours in SIOP 2001.

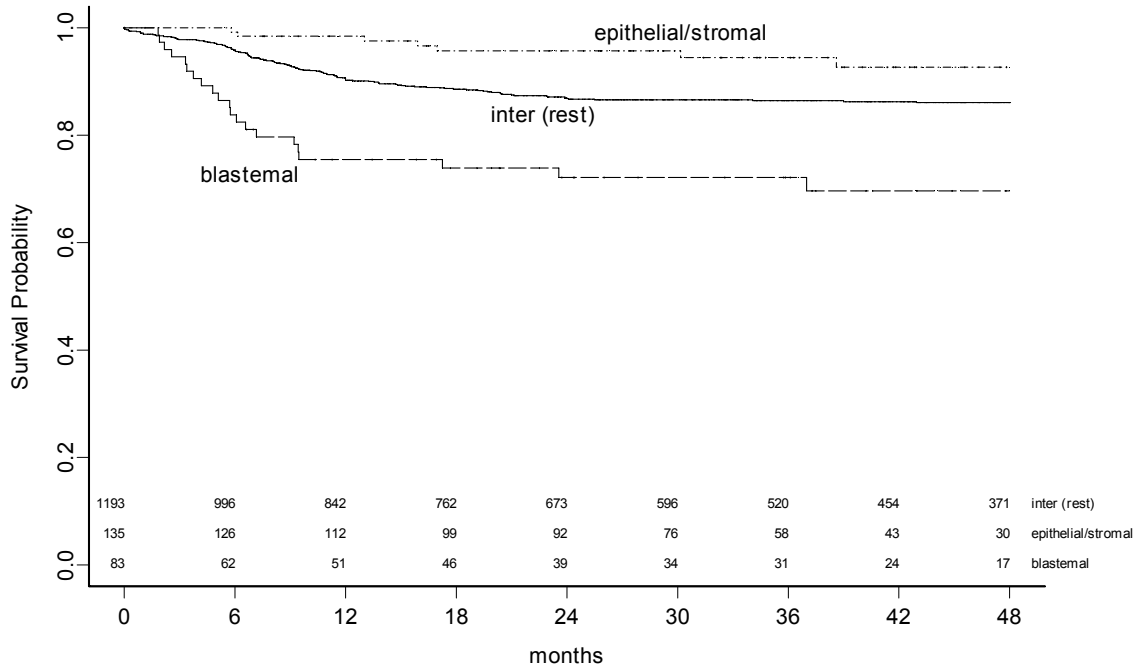


Figure 5. Event free survival including all stages for the different histological subtypes of the intermediate risk group. [Inter (rest) includes all intermediate risk tumours without epithelial, stromal and blastemal predominant; panel diagnosis].

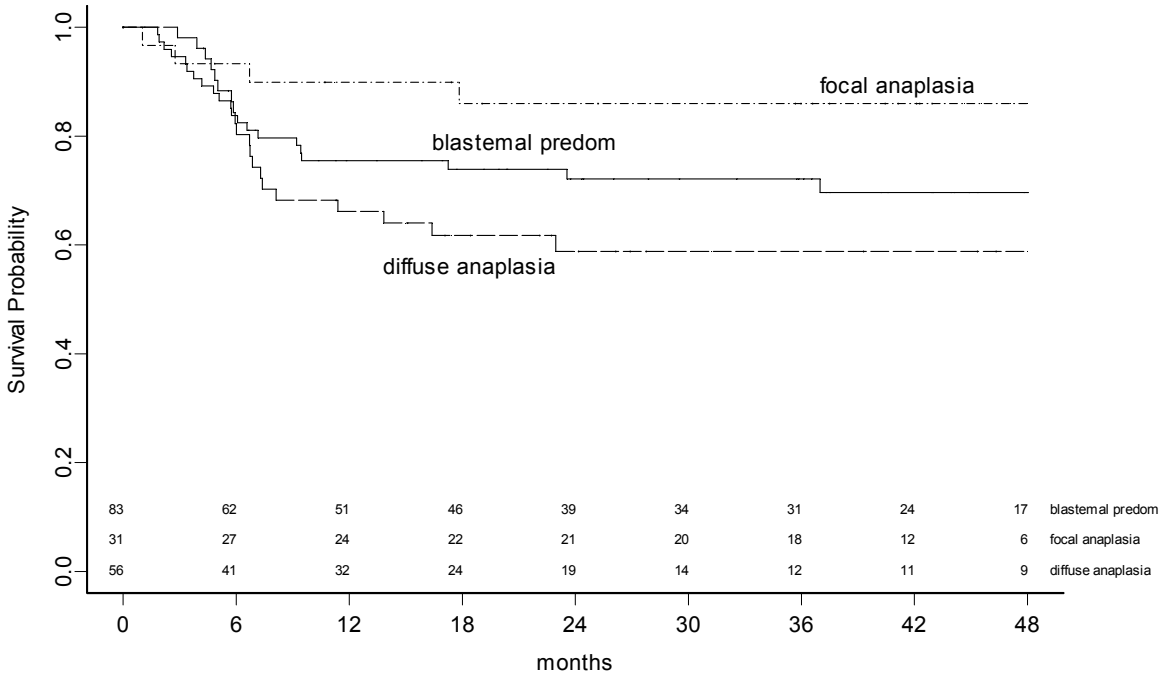


Figure 6. Event free survival for the blastemal predominat subtypes and focal vs diffuse anaplasia [all stages; panel diagnosis].

The prognosis for patients with clear cell sarcoma has improved. Treatment policy will be continued in SIOP 2001. The prognosis for patients with rhabdoid tumours is unchanged and still dismal. Further efforts are necessary.

II.2.4 Post-operative treatment

Post-operative treatment is given in all patients according to local stage and histology. Only patients receiving preoperative chemotherapy will be stratified according to the new histological classification.

In Germany tumour volume (> 500 ml) after pre-operative chemotherapy will also be used for stratification of postoperative treatment in intermediate risk tumours besides epithelial and stromal predominant histology. In all other countries a stopping rule is introduced to recognise a worse outcome of patients with tumours > 500 ml volume.

As a result of the randomised question of SIOP 93-01 the short arm of this trial will be used as postoperative treatment to all patients with stage I and an intermediate risk tumour according to the new definition, if they have received preoperative chemotherapy (38).

Patients with local stage II and III intermediate risk tumours after surgery and no metastasis at diagnosis will be randomised for postoperative treatment. The randomisation is between chemotherapy with or without Doxorubicin. This randomisation will reduce treatment intensity in about 1/3 of all eligible patients.

The event free survival for these patients in the SIOP 93-01 study is:

- **stage II**, 88% (80-92) at 2 years (C.I. 95%) and 88% (66-92) at 5 years
- **stage III**, 78% (66-86) at 2 years and 75% (49-84) at 5 years.

Treatment reduction is also given to all patients with tumours showing focal anaplasia.

Patients with a blastemal predominant subtype after preoperative chemotherapy will receive intensified postoperative treatment, because of their poor prognosis.

Postoperative chemotherapy recommended at ours for patients being primarily operated is given in chapter 8. They are based on UKCCSG and NWTSG protocols and survival data.

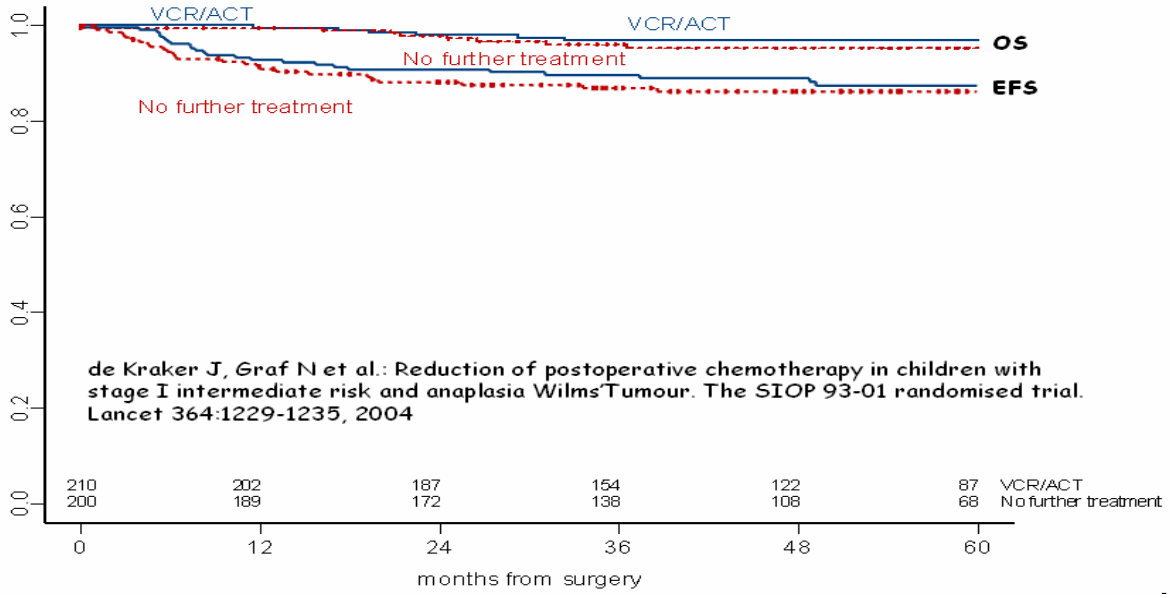


Figure 7. Effect of reduction reduction of postoperative chemotherapy in children with staghe I Wilms' Tunour.

II.2.5 Administration of Actinomycin D and Anthracyclin therapy

- ***Actinomycin D.*** In NWTS IV it was clearly shown that a single dose of actinomycin D of 45 µg/kg body weight is as efficient as giving actinomycin D in divided doses. This way of administering the drug is therefore recommended as the new standard, resulting also in less severe haematologic toxicity, and the requirement for fewer physician and hospital encounters (57).
- ***Anthracyclin therapy.*** Anthracyclines are potential cardiotoxic drugs. Cardiotoxicity is depending on the kind of the used drug, the dosage and the infusion duration. In SIOP 9 and 93-01 epirubicin was used with the exception of the German centers. A comparison regarding efficacy and toxicity of the two drugs will be done. Regarding early toxicity for adriamycin a retrospective analysis of GPOH patients from studies SIOP 9 and 93-01 was done. Posttherapy left ventricular fractional shortening was reduced in this analysis in 4 out of 157 (2.5%) patients. 2 of the 4 children had clinically reduced tolerance to exercise and received anticongestive therapy. Abnormal ECG findings that were not detectable prior to therapy were found in 7/124 children (101).

  This incidence is low and will hopefully not increase over time. Therefore it is possible to use the less expensive drug doxorubicin for all patients without increasing the risk of cardiotoxicity too much.

  A reduction of cardiotoxicity in SIOP 2001 will result by prolonging the infusion time from 4 to a minimum of 6 hours.

  Because cardiotoxicity is one of the most severe late effects, further reduction of this drug for patients with Wilms tumour is worthwhile. The better stratification of patients according to their histological subtype allows asking a randomised question of doxorubicin in the postoperative treatment of stage II and III intermediate risk patients. The risk of a poorer outcome of the "Non-Doxorubicin" arm is acceptable in this setting (101).

II.2.6 High risk protocol

Toxicity of the high risk protocol of SIOP 93-01 was high. In 60 % of GPOH patients receiving these protocol treatment violations were observed because of toxicity. Especially haematological toxicity was high. At least 4 patients did receive G-CSF after every cycle of the treatment.

Regarding the duration for administering the protocol, in nearly all patients' treatment was significantly prolonged. In 9 patients treatment was stopped before the end of the protocol. In four patients, because of progression of disease; and in 5 patients, because of toxicity. Figure 8 shows the duration of the protocol for the 82 documented high risk patients of GPOH.

Therefore the schedule of the high risk protocol is changed. VP16 and carboplatin are given over 3 days with reduced dosages (VP16: 5 x 100 mg/m² → 3 x 150 mg/m²; CARBO: 600 mg/ m² → 3 x 200 mg/m²).

Ifosfamide is substituded by cyclophosphamide, because of the potential risk of tubular damage of the remaining kidney. The dosage of the anthracycline is unchanged but the infusion time is prolonged.

Because treating patients according to the protocol ends up with a significant higher event free survival, the percentage of Protocol patients should be as high as possible.

This is also of importance, because of the fact, that treatment reduction might be at an edge, where further reduction can harm patients.

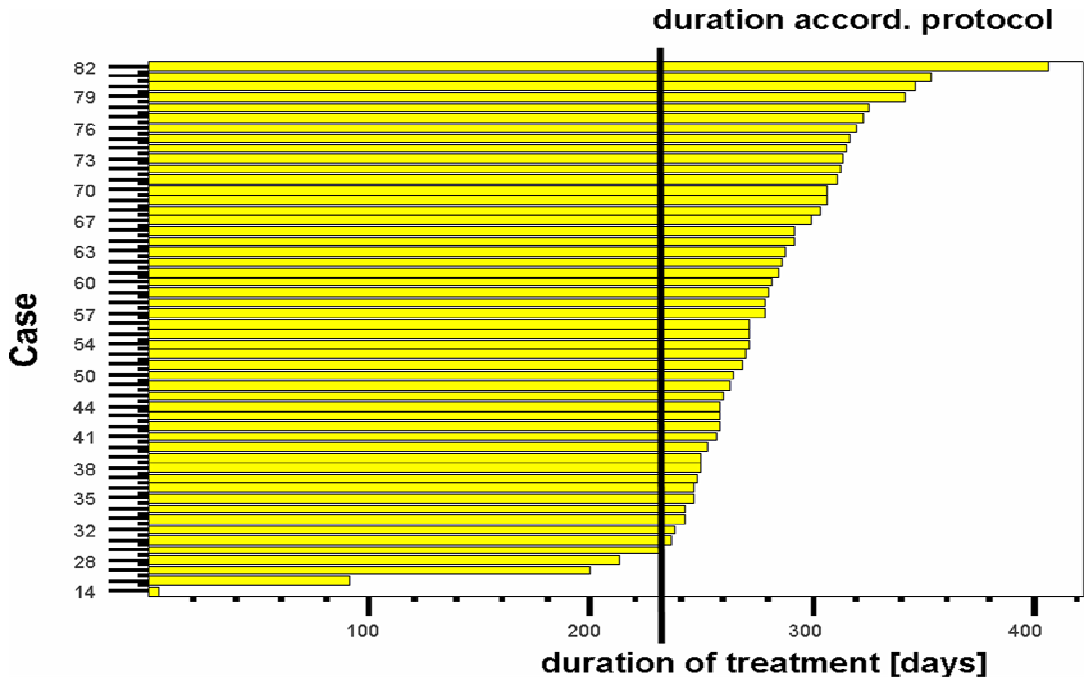


Figure 8. Duration of the high risk protocol in 82 GPOH patients.

II.3 Rational & Objectives of ACGT Nephroblastoma Trial

II.3.1 Introduction

This is a critical time in the history of cancer research as recent advances in methods and technologies have resulted in an explosion of information and knowledge about cancer and its treatment. As a result, our ability to characterize and understand the various forms of cancer is growing exponentially.

Information arising from post-genomics research, and combined genetic and clinical trials on one hand, and advances from high-performance computing and informatics on the other is rapidly providing the medical and scientific community with new insights, answers and capabilities. The breadth and depth of information already available in the research community at large, present an enormous opportunity for improving our ability to reduce mortality from cancer, improve therapies and meet the demanding individualization of care needs

A future healthcare system emerges from the envisioned and raising genomic medicine era. It will be a more *individualised* or *personalised* system. At the same time it designates the needed and inevitable investments in technological advances towards its realisation and achievement. The needs and and benefits of the future healthcare reveals the motivation of the *ACGT* project.

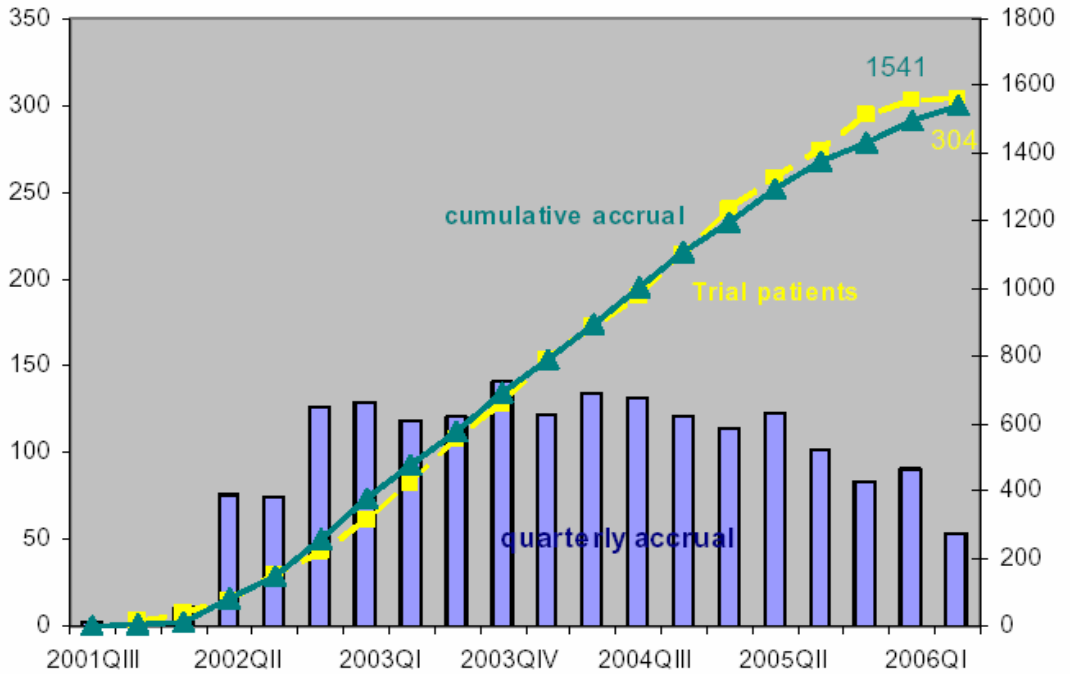
II.3.2 Relation between SIOP 2001 and ACGT

The SIOP 2001 study and trial is running in many European countries and outside, like Brazil for treating children and young adults with nephroblastoma. The trial was opened

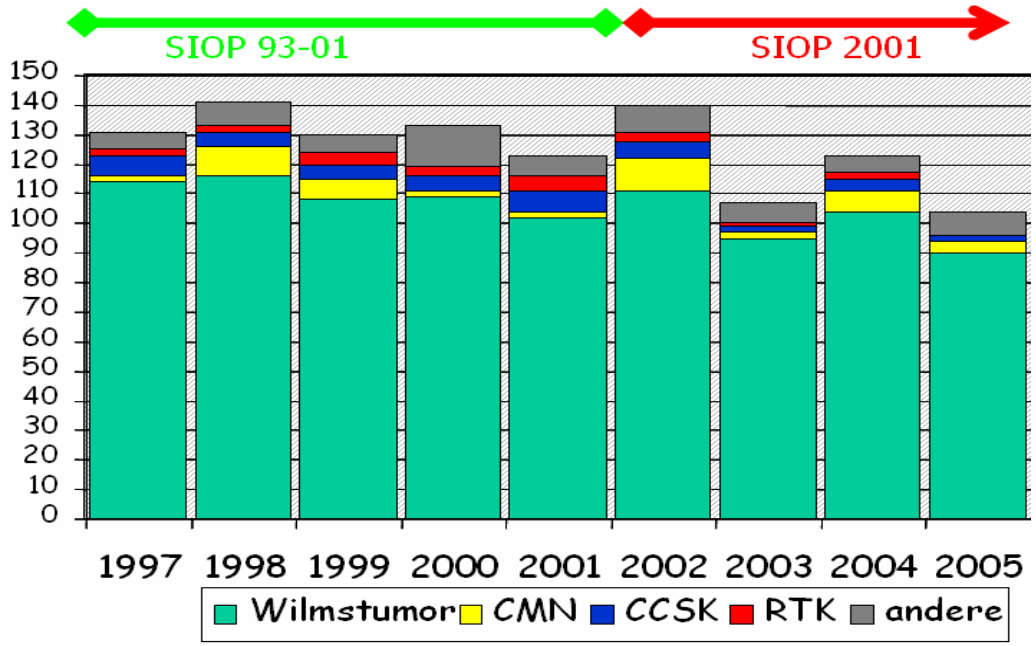
in 2001. Since that time 1818 patients have been enrolled. About 25 % of included patients are registered in Germany (6th interim report, April 2006, Amsterdam). Up to that time 314 patients have been randomized.

	2001	2002	2003	2004	2005	2006	All N	All %
GCBTTW	1	43	78	76	44	6	248	14
GPOH	5	139	104	122	80	11	461	25
SFCE		52	86	86	72	3	299	16
SIOP-NL	8	115	133	110	90	14	470	26
UKCCSG		56	96	92	84	12	340	19
All	14	405	497	486	370	46	1818	100

The quaterly accrual of patients entered into the SIOP 2001 study and trial is shown in the following figure:



The accrual rate of patients in Germany is shown in the following figure.



The main difference between SIOP 2001 and the ACGT Nephroblastoma trial is the addition of scenarios outlined in this protocol (chapter 15, and 16.6). Inclusion criteria, diagnostic procedures, treatment, randomisation and primary and secondary study and trial questions are exactly identical with the exception of non including bilateral tumours into the ACGT trial. The reason for that is, the individualized preoperative chemotherapeutic phase, that makes the correlation to the given specific timepoints in the scenario for the detection of an humoral immune response highly difficult. This is especially true, because of the low number of bilateral tumours (about 5 % of all patients).

A further difference is, that with the beginning of the ACGT nephroblastoma trial only patients from Germany will be included. The inclusion of patients outside from Germany will be asked, after first preliminary results are available. The scenario will be presented together with ACGT at the committee meeting of the SIOP nephroblastoma trial, which will be held together with the biological subcommittee of the trial in January 2007 in London.

Together with WP 10 in ACGT a model for the prediction of the preoperative chemotherapeutic response will be developed.

The main scenario in the ACGT nephroblastoma trial is the identification of nephroblastoma antigens and the determination of the seroreactivity.

A second scenario is the scenario for reporting of adverse events (AEs) and severe adverse reactions (SARs).

II.3.3 Purpose and Future of the ACGT Nephorblastoma trial

The ultimate objective of the ACGT project is the provision of a unified technological infrastructure which will facilitate the seamless and secure access and analysis, of multi-level clinico-genomic data enriched with high-performing knowledge discovery operations and services.

ACGT's vision is to become a pan-European voluntary network or grid connecting individuals and institutions to enable the sharing of data and tools, creating a European

Wide Web of cancer clinical research. The ultimate goal is to speed the delivery of innovative approaches for the prevention and treatment of cancer. The infrastructure work in the ACGT nephroblastoma trial is based on the the ACGT main components, that include biomedical technology grid layer, data access and applications, data mining and knowledge discovery tools, ontologies and semantic mediation tools, technologies and tools for in-silico oncology, the grid-enabled application layer, the integrated *ACGT* environment. All the interoperable tools that will be developed will have the benefits of open access to share data and standards to the Cancer Research Community.

The ACGT nephroblastoma trial will be "used" as a clinical trial in this sense and will run in the developing platform of ACGT

To run the ACGT nephroblastoma trial ontology based clinical database with remote data entry and access will be developed. This database will be build-up in a modular way. The purpose is that it can easily be transferred to other investigator initiated clinical trials. Available standards will be incorporated; ethical and legal issues will be recognized. Tools for reporting SAEs and SUSARs will be integrated. All will be based on the Grid architecture of the ACGT platform.

The actual ACGT nephroblastoma trial will be used as a test for investigator initiated clinical trials. It is the declared intention that the next SIOP nephroblastoma trial should be an ACGT trial. For that reason ACGT will be promoted within the international SIOP nephroblastoma committee and also the biological subcommittee of the trial.

II.3.4 Nephroblatoma Study Design and Patient Selection

II.3.4.1 All unilateral Wilms Tumors

At diagnosis all patients with a unilateral Wilms Tumor have to be registered. At this time they are divided into:

- localized disease patients
- metastatic disease patients

- *Localized disease patients.* These patients are eligible for the protocol if:
  1. All ages
  2. Unilateral tumour with clinical and ultrasonic characteristics compatible with nephroblastoma or with a biopsy proven histological diagnosis.
  3. No previous anti-tumour treatment.
  4. No metastasis.
  5. Written informed consent and ethical committee approval.
- *Metastatic disease patients.* These patients are eligible for the protocol if:
  1. All ages
  2. Unilateral tumour with clinical and ultrasonic characteristics compatible with nephroblastoma or with a biopsy proven histological diagnosis.
  3. No previous anti-tumour treatment.
  4. Presence of metastatic disease.
  5. Written informed consent and ethical committee approval.
- *Protocol patients.* All localized and metastatic patients that fulfil the criteria to be eligible for the SIOP 2001 protocol. They will be treated according to the treatment principles set out in this protocol.

- *Trial patients.* These are protocol patients that turn out to be stage II / III, intermediate risk histology, at or shortly after the operation and are randomized to receive a treatment according to one of the randomization arms.

II.3.4.2 Study patients / Exclusion criteria

All other patients that do not fulfil the eligibility criteria of the protocol and those for whom there is no strict protocol available are study patients. They are excluded from the protocol, but they will be followed in respect to survival and reason for death in case they have died. Analysis of Antigen pattern is possible.

More precisely these exclusion criteria are:

- ✓ Bilateral tumours with or without metastases.
- ✓ Renal tumours other than Wilms tumour diagnosed at registration.
- ✓ Patients who are unable to follow the protocol for reasons of associated pathology or social, geographical problems or when follow-up is not possible.
- ✓ Patients who have already been given radiotherapy or chemotherapy other than stated in the protocol before surgery or patients referred to the centre after surgery.
- ✓ Patients referred for treatment of recurrent disease.

II.3.5 SIOP 2001 and the ACGT Nephroblastoma Trial

All patients enrolled in the ACGT Nephroblastoma Trial are also patients of the Nephroblastoma Trial SIOP 2001/GPOH. Only patients enrolled in the ACGT Nephroblastoma trial will be analysed for humoral immune response against nephroblastoma specific antigens according to the scenario described in the sequel.

II.3.5.1 Pretreatment Investigations

The following investigations are the minimum required observation and the results should be recorded in the patient's personal files.

- **Physical examination**
  1. Weight and height
  2. Side and size of the tumour (see also ultrasonography)
  3. Size of the liver (axillary/mammilla/midline)
  4. Blood Pressure
  5. Suspect lymph nodes or other masses
  6. Congenital anomalies (e.g. aniridia, hemihypertrophy, urogenital malforms and other).

- **Laboratory examination**
  1. Hemoglobin, Hematocrit
  2. WBC and platelet count
  3. Blood chemistry: serum creatinine + centers own protocol.
  4. Urinanalysis: Presence or absence of protein, white and red cells. Verify that urinary excretion of catecholamine (HVA, VMA, DOPA) is normal.

- **Imaging studies**

  - ***Ultrasonic examination of abdomen is mandatory.*** It is simple, fast and non invasive. It can help distinguish between a cyst and a tumour and is very helpful in detecting small tumours on the opposite kidney, tumour thrombi in the inferior vena cava, liver and abdominal metastases. Size of the renal mass, in three dimensions, should be measured before initial treatment and just before surgery. It is the first choice investigation in case of suspected WT. One should try to measure just the tumour and not the whole kidney. It should be clearly stated how the tumour was measured.

    Separate masses should be measured separately and this should be clearly stated on the forms. The pre-operative measurements should be taken one day before surgery and correlated to the weight of the specimen.

    a = length (cm)
    b = width (cm) **V = a x b x c x 0,523 in cm^3**
    c = thickness (cm)
    V= volume

  - ***CT-scan of the abdomen.*** Maybe helpful if ultrasound gives insufficient information and in case of doubtful anomalies in the contralateral kidney or liver.

  - ***P.A. and lateral views of the chest*** should be done as a routine in order to detect pulmonary metastases. CT-scan only in case of doubt.

  - ***Selective renal arteriography*** should only be done preferably just before surgery by a radiologist skilled in performing the examination in young children to document size and site of the tumour in bilateral cases, in horse-shoe kidney tumours and other situations in which the surgeon needs that information.

  - ***Needle biopsy*** performed by an experienced person posteriorly taken is permitted without changing the stage.

  - ***Echocardiography*** measuring the percentage of contraction of the diameter of the left ventricle is mandatory in intermediate risk, stage II and III patients and all high risk patients. This investigation should be done before the first administration and with regular intervals starting after a cumulative dose of 200 mg/m^2 and thereafter at the end of therapy. From there on once yearly for at least 10 years.

⇨ **Documentation in stage IV patients.** All stage IV patients are protocol patients. Initial detailed and quantitative evaluation of the extension of the disease should be performed and mentioned on the form. CT scan of the site of the metastases is optional.

II.3.6 A Practical Guide to Diagnosis

	Investigation	Reasons
STEP-1	1. Start to collect 24hrs. Urine while on a stan-dard-ized diet.	To exclude neuroblastoma with-out inter-fer-ence by con-trast medium. To establish kidney func-tion and test for haematuria and tumour cells.
	2. Ultrasound	To make 3 dimensional measurements. To establish if this is an intra-renal pro-cess. To investigate the opposite kid-ney. Is it cystic, so-lid or both? To search for other abnormalities in the abdomen. Intravascular extension, etc.
	3. Plain X-ray of the chest in 2 directions	To exclude pulmonary metastases.

IF NO SOLID DIAGNOSIS YET

	Investigation	Reasons
STEP-2	CT-scan abdomen if appropriate	To confirm intrarenal lesion with certain characte-ristics, relations to other structures. Lymph nodes, invasion of vessels, other organs and structures. Also useful for measurements.
	CT-scan thorax if any doubt on chest x-ray	To further exclude metastases or to confirm.

IF STILL NO SOLID DIAGNOSIS YET

	Investigation	Reasons
STEP-3	Needle Biopsy	To confirm diagnosis by histology consult the recommendations for this procedure.

II.3.7 Use of Needle Biopsy in Renal Tumours in Children

Consider needle biopsy in case of:

- ***Unusual clinical presentations***:Age > 5-6 years; Urinary infection; Septicaemia; Psoas inflammation
- ***Unusual findings by imaging***:Calcification**;** Voluminous adenopathies**;** Renal parenchyme not visible**;** Almost total extrarenal process**;** Pure cystic structures
- ***Contraindications for the use of Needle Biopsy***: Elevations of urinary catecholamines**;** Age < 6 months**;** Suspicion of rupture or hemorrhage
- ***PROCEDURE***:
  - ✓ Normal blood coagulation tested;
  - ✓ General anaesthetic;
  - ✓ All retroperitoneal tumours should be biopsied from posterior side;
  - ✓ Ultrasound guided biopsy preferably;
  - ✓ Pathologist at hand for tissue handling;
  - ✓ Discuss with your local radiology on coaxial needle technique.

Humoral immune response against nephroblastoma specific antigens:

Serum of patients will be investigated at 4 different timepoints for humoral immune response against nephroblastoma specific antigens. These timepoints are defined in the following way:

- ⇨ **timepoint 1** (t1): at **diagnosis**
- ⇨ **timepoint 2** (t2): **after preoperative chemotherapy** and before surgery
- ⇨ **timepoint 3** (t3): **after surgery**
- ⇨ **timepoint 4** (t4): at the **end of treatment**

In patients without preoperative chemotherapy but immediate tumornephrectomy t2 does not exist. In this situation t1 will be the timepoint at diagnosis and before surgery. Native Blood without any manipulation and cooling has to be send directly to the study center in Homburg (Prof. Dr. N. Graf). The analysis will be done in the laboratory of Prof. Dr. Meese, University of the Saarland.

II.3.8 Therapy Protocols for Localized Disease

	STAGE I	STAGE II	STAGE III
LOW Risk	NO FURTHER TREATMENT	AV-2	AV-2
INTERMEDIATE Risk	AV-1	R → DOX / DOX -	R → RT / DOX + / RT / DOX -
HIG Risk	AVD	HIGH RISK + RT	HIGH RISK + RT

Copy this form and put in your patients file!!!

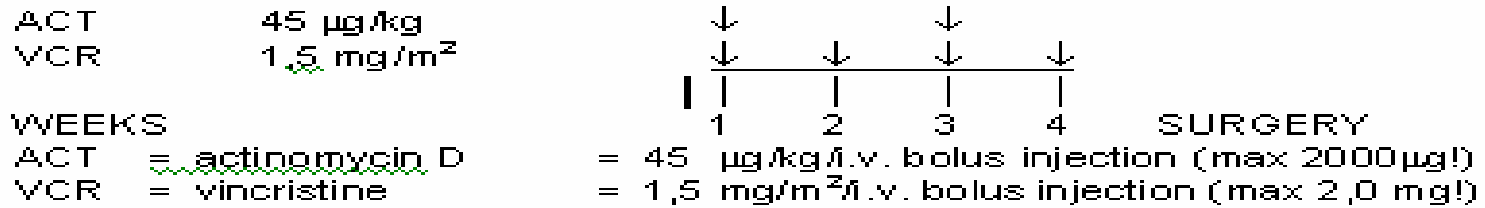
LOCALIZED DISEASE

ESSENTIAL OBSERVATIONS AND THEIR TIME SCHEDULE

								LOCAL	NATIONAL	PANEL	
ACT	45 µg/kg	↓		↓		SURGERY		PATHOLOGIST	REVIEW	REVIEW	
VCR	1,5 mg/m^2	↓	↓	↓	↓	⇓		⇓	⇓		
WEEKS		1	2	3	4	5		6	7		
Sonography. Measurement in 3 directions of tumour and detailed report		♦		♦		♦					
Blood for immune response		♦				♦	♦				♦
Chest X-ray, abdominal CT or MRI		♦				♦					
Surgical report. Specimen + histological form immediately to pathologist. Sampling of material for biological studies							♦				
Diagnosis								♦			

LOCALIZED DISEASE

PRE-OPERATIVE TREATMENT

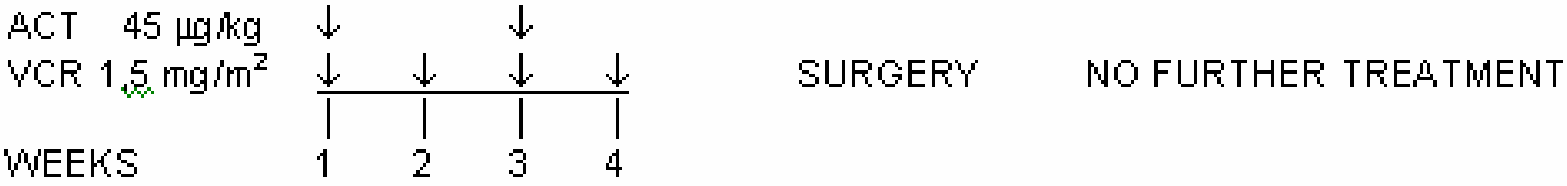


ACT = actinomycin D = 45 µg/kg/i.v. bolus injection (max 2000µg!)
VCR = vincristine = 1,5 mg/m²/i.v. bolus injection (max 2,0 mg!)

If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

STAGE I, LOW RISK

PRE-OPERATIVE TREATMENTPOST-OPERATIVE TREATMENT



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VCR = vincristine = 1,5 mg/m²/i.v. bolus injection (max 2,0 mg!)

If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses should be reduced to 2/3

STAGE I, INTERMEDIATE RISK

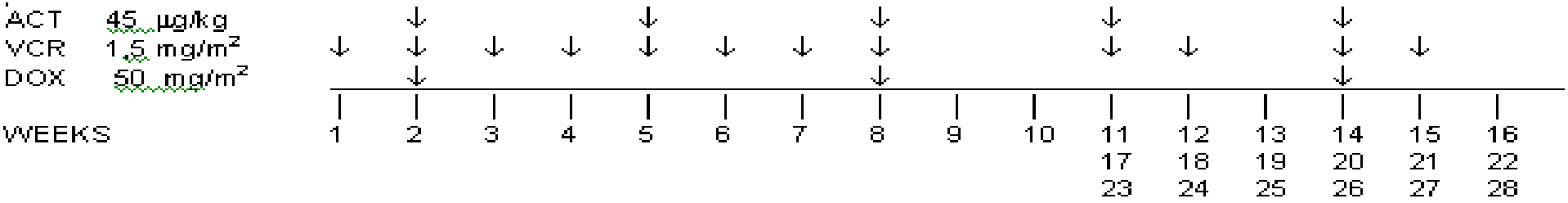
PRE-OPERATIVE TREATMENT -- POST-OPERATIVE TREATMENT

ACT = actinomycin D = 45 µg/kg/i.v. bolus injection (max 2000 µg!)
VCR = vincristine = 1,5 mg/m²/i.v. bolus injection (max 2,0 mg!)

If body weight < 12 kg: dose reduction to 2/3 for each drug.
Major intolerance: doses on the next course should be reduced to 2/3

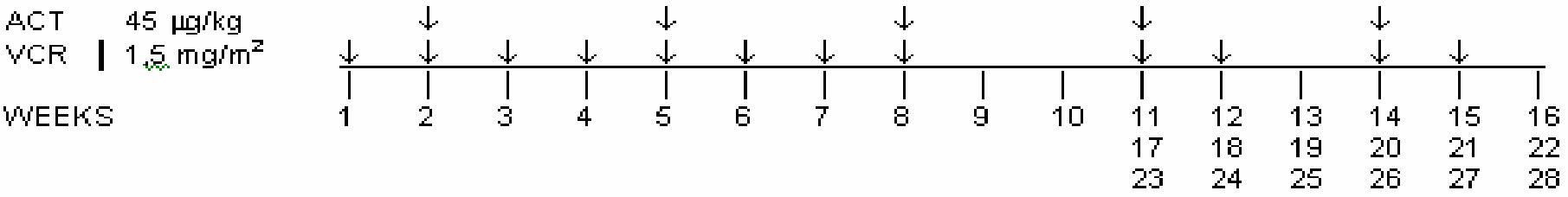
STAGE I, HIGH RISK

POST-OPERATIVE TREATMENT, REGIMEN AVD



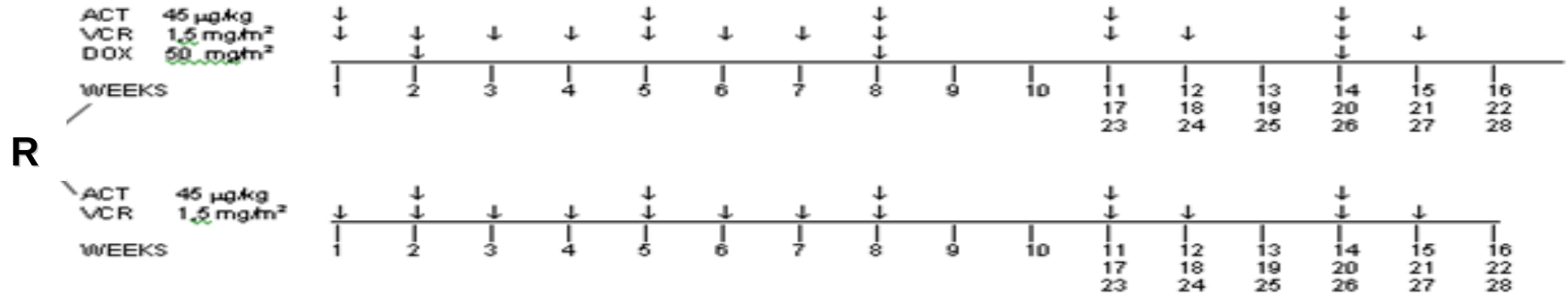
ACT = actinomycin D = 45 µg/kg/ i.v. bolus injection (max 2000 µg!)
VCR = vincristine = 1,5 mg/m²/ i.v. bolus injection (max 2,0 mg!)
DOX = doxorubicin = 50 mg/m²/ i.v. in 4 - 6 hours
If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

STAGE II, LOW RISK
POST-OPERATIVE TREATMENT



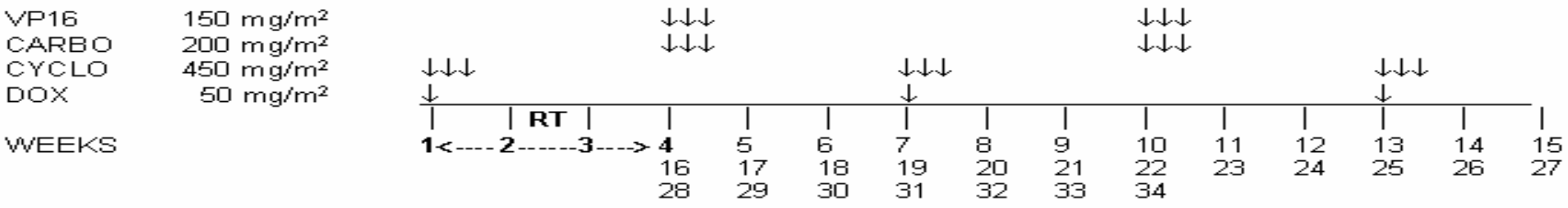
ACT = actinomycin D = 45 µg/kg/ i.v. bolus injection (max 2000 µg!)
VCR = vincristine = 1,5 mg/m²/ i.v. bolus injection (max 2,0 mg!)
If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

STAGE II, INTERMEDIATE RISK
POST-OPERATIVE TREATMENT



ACT = actinomycin D = 45 µg/kg/ i.v. bolus injection (max 2000 µg!)
VCR = vincristine = 1,5 mg/m²/ i.v. bolus injection (max 2,0 mg!)
DOX = doxorubicin = 50 mg/m²/ i.v. 6 hours
If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

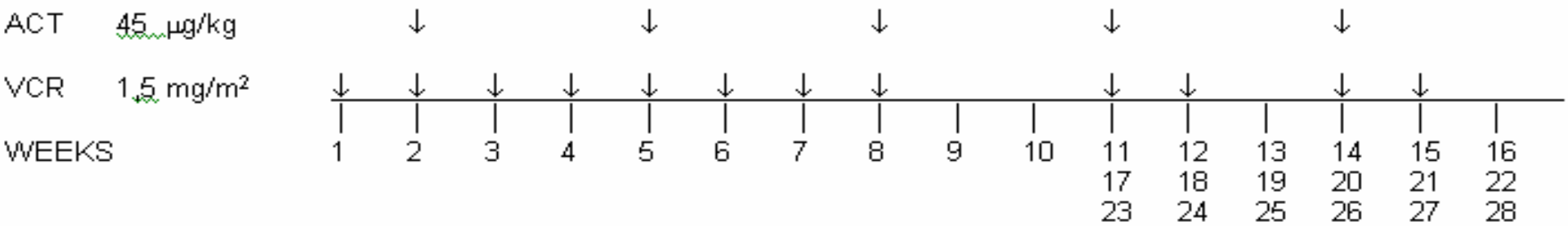
STAGE II, HIGH RISK -- POST-OPERATIVE TREATMENT



VP16 ≡ etoposide = 150 mg/m²/i.v./in 1 hour
CARBO ≡ carboplatin = 200 mg/m²/i.v./in 1 hour
CYCLO ≡ cyclophosphamide = 450 mg/m²/i.v./in 1 hour
DOX ≡ doxorubicin = 50 mg/m²/i.v./in 6 hours, just before the first cyclo administration

If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

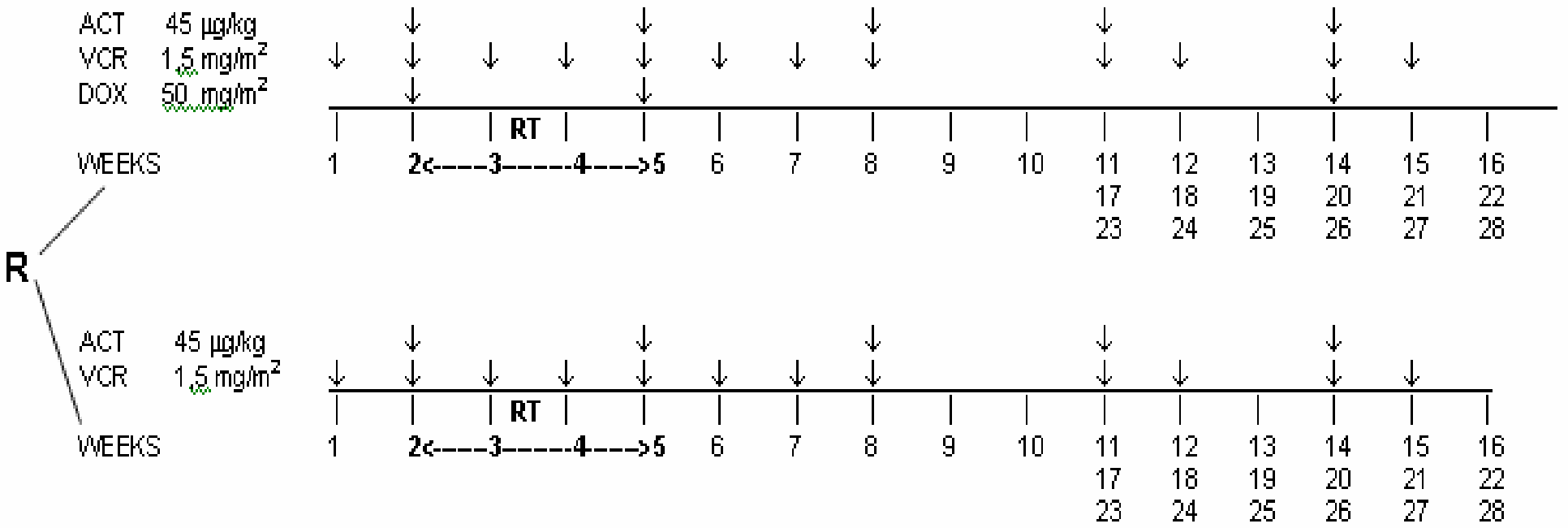
STAGE III, LOW RISK -- POST-OPERATIVE TREATMENT



ACT = actinomycin D = 45 µg/kg/i.v. bolus injection (max 2000 µg!)
VCR = vincristine = 1,5 mg/m²/i.v. bolus injection (max 2,0 mg!)

If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

STAGE III, INTERMEDIATE RISK -- POST-OPERATIVE TREATMENT



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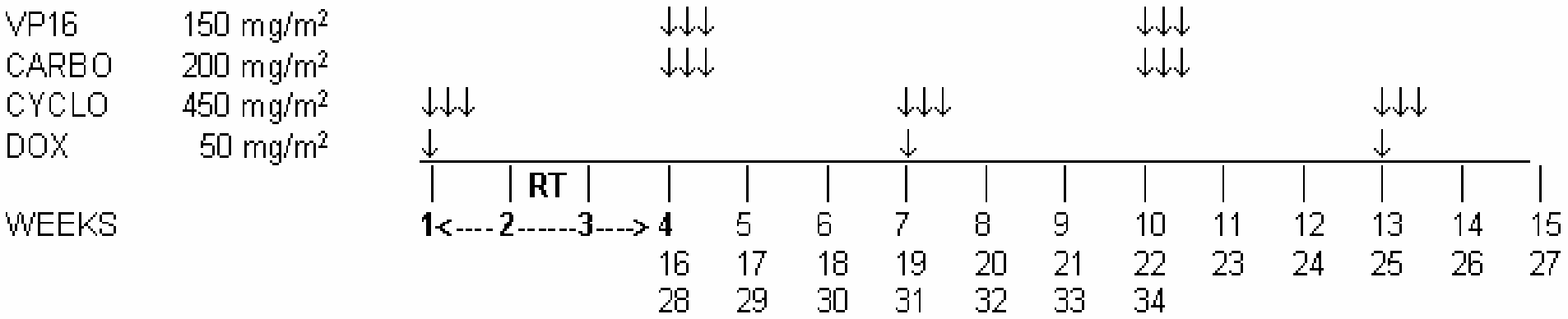
VCR = vincristine = 1,5 mg/m²/ i.v. bolus injection (max 2,0 mg!)
DOX = doxorubicin = 50 mg/m²/i.v. 6 hours
If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

STAGE III, HIGH RISK -- POST-OPERATIVE TREATMENT



VP16	= etoposide	= 150 mg/m²/i.v./in 1 hour
CARBO	= carboplatin	= 200 mg/m²/i.v./in 1 hour
CYCLO	= cyclophosphamide	= 450 mg/m²/i.v./in 1 hour
DOX	= doxorubicin	= 50 mg/m²/i.v./in 6 hours, just before the first cyclo administration

If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

II.3.9 Therapy Protocol for Metastatic Disease

All metastatic sites are included in the study. Initial detailed and quantitative evaluation of the extension of the disease should be performed and mentioned in the form.

Pulmonary metastasis are documented with a plain X-ray of the chest. CT-scan is used if there is any doubt about the presence of metastasis. Those centres that use CT-scan of the thorax as routine work-up are of course allowed to do so. All the other sites are documented in the most relevant way.

II.3.9.1 Pre-operative treatment

- The three drug schedule with Vincristine, Actinomycin D and Doxorubicin will be used. Duration of pre-operative treatment is 6 weeks.
- Thereafter surgery for the primary tumour is performed.
- As soon as possible after the surgical procedure for the renal tumour the metastatic side will be evaluated.

II.3.9.2 Post-operative treatment

From this point on the treatment protocol will be determined by the local stage of the abdominal tumour, the histological type of this tumour and the result of the evaluation of the metastatic side(s).

- For pulmonary metastases there are three starting points:
  - **A.** Metastasis absent or completely removed by the surgeon.
  - **B.** Metastasis incompletely removed or multiple inoperable metastases
  - **C.** Patients with high risk histology of the primary tumour.

Treatment guidelines are given on the following pages.

- For non pulmonary metastasis the treatment principle is the same.

The responsible doctor will have to work this out for forthcoming situations.

STAGE IV -- PRE-OPERATIVE TREATMENT

ACT	45 µg/kg	↓			↓			↓				
VCR	1,5 mg/m²	↓		↓	↓		↓	↓		↓		
DOX	50 mg/m²	↓						↓				
		|		|		|		|		|		
WEEKS		1		2	3		4	5		6		SURGERY

ACT = actinomycin D = 45 µg/kg/i.v. bolus injection (max 2000 µg!)
VCR = vincristine = 1,5 mg/m²/i.v. bolus injection (max 2,0 mg!)
DOX = doxorubicin = 50 mg/m²/i.v./in 6 hours
If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3

STAGE IV -- POST-OPERATIVE TREATMENT

A. METASTASES ABSENT OR COMPLETELY RESECTED BY THE SURGEON

Local stage I and II: Local stage II treatment, three drugs arm
Local stage III: Local stage III treatment, three drugs arm + flank irradiation

B. METASTASES INCOMPLETELY RESECTED OR MULTIPLE INOPERABLE

Local stage I and II: High risk treatment, no abdominal irradiation

Evaluate the status of the pulmonary metastases at week 9:
- if CR → no pulmonary irradiation
- if no CR → pulmonary irradiation

In anyother situation, please contact the study center.

Local stage III: High risk treatment, with abdominal irradiation

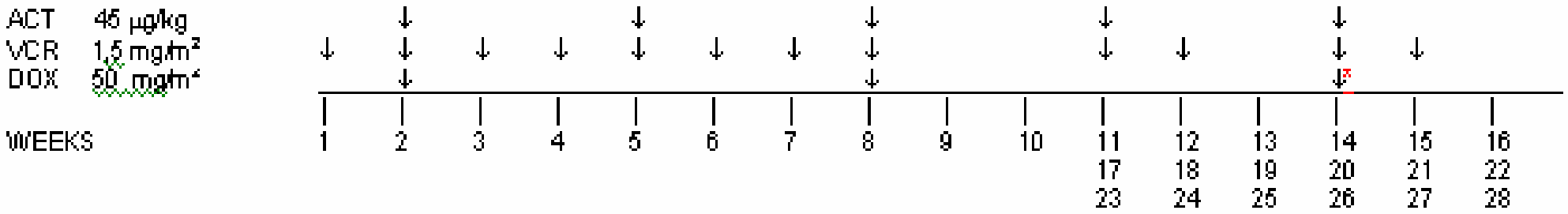
A. HIGH RISK PRIMARY TUMOUR

Local stage I: High risk treatment. No abdominal irradiation. Pulmonary irradiation
Local stage II and III: High risk treatment with abdominal and pulmonary irradiation

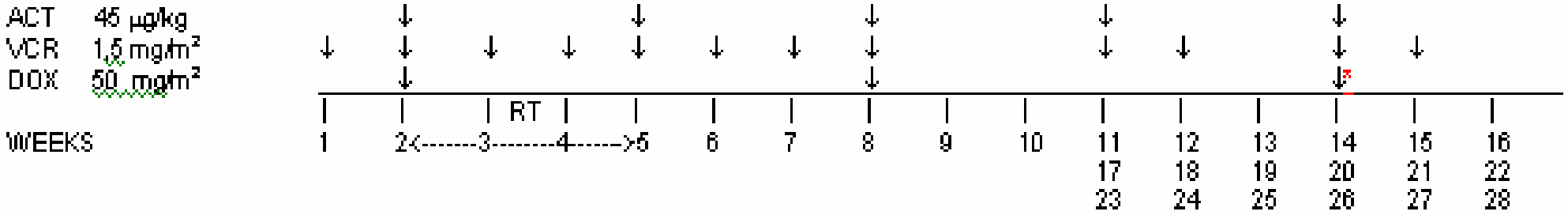
STAGE IV, POST-OPERATIVE TREATMENT, Group A

A. METASTASES ABSENT OR COMPLETELY RESECTED BY THE SURGEON

LOCAL STAGE I & LOCAL STAGE II



LOCAL STAGE III

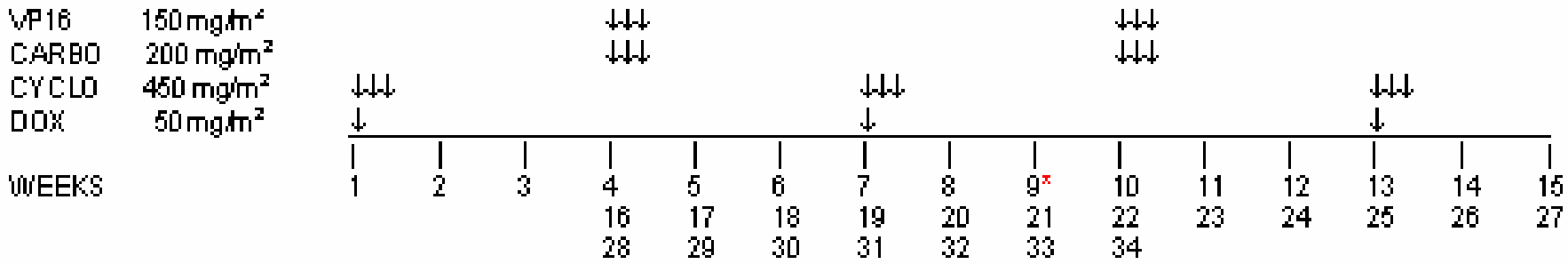


If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3
During irradiation: dose reduction to 2/3 for each drug
* no DOX in week 26

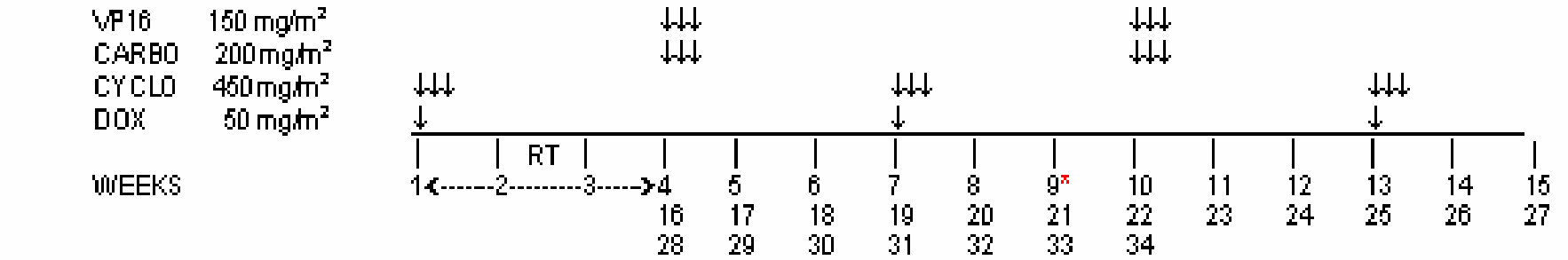
STAGE IV, POST-OPERATIVE TREATMENT, Group B

B. METASTASES INCOMPLETELY RESECTED OR MULTIPLE INOPERABLE

LOCAL STAGE I-II, NO ABDOMINAL IRRADIATION



LOCAL STAGE III, WITH ABDOMINAL IRRADIATION

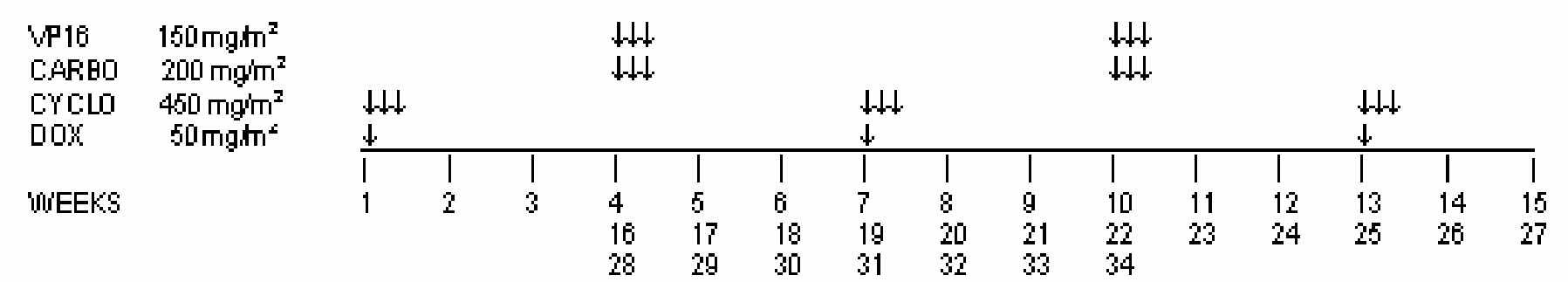


If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3
During irradiation: dose reduction to 2/3 for each drug
* pulmonary irradiation, evaluate at week 9 and decide on pulmonary irradiation

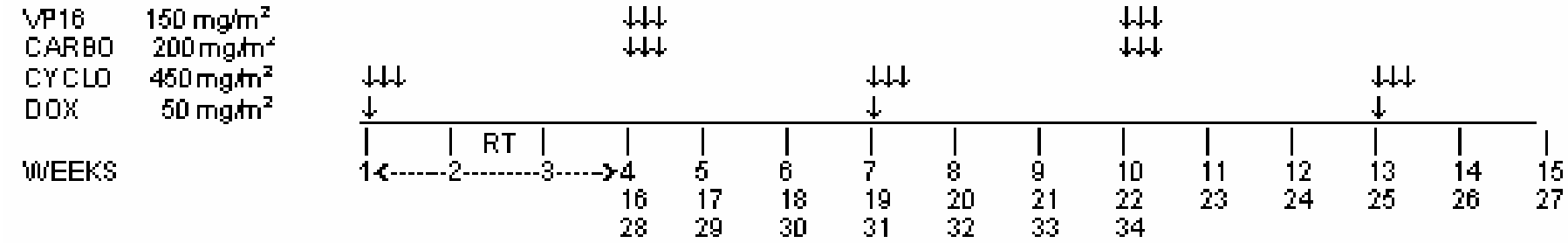
STAGE IV, POST-OPERATIVE TREATMENT, Group C

C. HIGH RISK PRIMARY TUMOUR

LOCAL STAGE I, NO ABDOMINAL IRRADIATION, WITH PULMONARY IRRADIATION



LOCAL STAGE II AND III, WITH ABDOMINAL AND PULMONARY IRRADIATION



If body weight < 12 kg: dose reduction to 2/3 for each drug
Major intolerance: doses on the next course should be reduced to 2/3
During irradiation: dose reduction to 2/3 for each drug

II.3.10 Immediate Nephrectomy: Recommended Chemotherapy

Risk group assignment in cases treated with immediate nephrectomy is based solely on tumour stage and presence of unfavourable histology (ie anaplasia).

The SIOP pathological risk group B applies to tumours that have not received pre-operative chemotherapy. Note that the presence of large amounts of viable blastema is of no prognostic significance in immediate nephrectomy specimens.

The following recommendations are based on the United Kingdom experience in UKW 1 & 2 studies) (117, 105). Modifications to the length of therapy, total dose of anthracycline and treatment of focal anaplasia have been made in the light of published data from NWTS 4 study (57, 60).

- ***Staging.*** Definition of tumour stage will be as for tumours receiving pre-operative chemotherapy except that the concept of "regressive changes/necrotic tumour" will not be applicable. It is of particular importance to assign stage I and stage II correctly, as these patients receive reduced chemotherapy compared to previous SIOP protocols. Lymph nodes must be adequately sampled at time of nephrectomy (see surgical guidelines).

II.3.11 Post-operative chemotherapy regimens for primary excision tumours

- *Regimen 1 (intensive VCR): Stage I, intermediate risk (excluding focal anaplasia).* Vincristine 1.5 mg/m^2 (maximum dose 2 mg) weekly for 10 weeks (10 doses in total). The first dose is to be given once peristalsis is established following surgery. Note: **infant doses are lower**. Total duration of therapy: 10 weeks.

- *Regimen 2 (AV): Stage II, low and intermediate risk and Stage I, focal anaplasia.* Vincristine 1.5 mg/m^2 (maximum dose 2 mg) weekly for 11 weeks and then three weekly, at weeks 14, 17, 20, 23 and 26 (16 doses in total), plus: → Actinomycin D 45µg/kg (maximum dose 2 mg) at weeks 2, 5, 8, 11, 14, 17, 20, 23 and 26 (9 doses in total). Note: **infant doses are lower**. Total duration of therapy: 26 weeks.

- *Regimen 3 (sequential AVD): Stage III low and intermediate risk. Low risk: no radiotherapy.* Vincristine 1.5 mg/m^2 (maximum dose 2 mg) weekly for 10 weeks and then three weekly, at weeks 13, 16, 19, 22, 25 and 28 (16 doses in total), plus: → Actinomycin D 45µg/kg (maximum dose 2 mg), 50% dose at week 2* then full dose at weeks 10, 16, 22, 28 (5 doses in total) → Doxorubicin 50 mg/m^2 at weeks 7, 13, 19, 25 (4 doses (200 mg/m^2) in total). Each dose to be infused over 4 hrs minimum → Abdominal radiotherapy (15 Gy) to be given weeks 2 – 4. Total duration of therapy: 28 weeks.

- *Regimen 4 (VCCD): Stage IV (after Non-CR after AVD) low and intermediate risk.* VP16 150 mg/m^2/d/x3 at weeks 4, 10, 16, 22, 28, 34 (18 doses in total), plus: → Carboplatin 200 mg/m²/dx3 at weeks 4, 10, 16, 22, 28, 34 (18 doses in total) → Cyclophosphamid 450 mg/m²/d/x3 at weeks 1, 7, 13, 19, 25, 31 (18 doses in total), plus: → Doxorubicin 50 mg/m²/d, 6 hour infusion at weeks 1, 7, 13, 19, 25, 31 (18 doses in total). Total duration of therapy: 34 weeks. Stage IV patients having immediate nephrectomy should be few in number and confined to patients presenting as surgical emergencies with unrecognised lung or liver metastases. They should be treated with the three drug "preoperative" chemotherapy for stage IV tumours Metastatic response should be evaluated at week 6 by CXR. Subsequent chemotherapy is dictated according to whether or not metastatic complete remission has been achieved by chemotherapy +/- surgery, as per the main protocol recommendations, i.e. complete responders continue the three drug regimen; incomplete responders switch to the high risk post-operative regimen.

II.3.12 Post-operative chemotherapy for high risk histology tumours having primary excision:

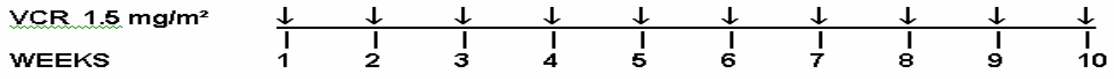
- ***Focal anaplasia***
  Stages I & II regimen 2 (AV), i.e. 2 drugs (VCR and ActD x 26 weeks)
  Stages III & IV sequential AVD according to regimen 3 or 4, depending on requirement for radiotherapy.

- ***Diffuse anaplasia***
  Stage I regimen 4 (sequential AVD). No RT
  Stages II-IV SIOP 'high risk' post operative chemotherapy plus RT.

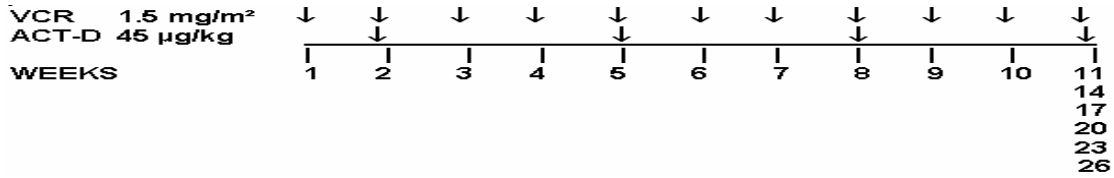
- ***CCSK***
  Stage I – IV SIOP high risk post operative chemotherapy
  No RT for stage I, RT for all other stages (dose and # as per intermediate risk WT)

II.3.13 Flow diagrams for recommended chemotherapy for cases receiving immediate nephrectomy

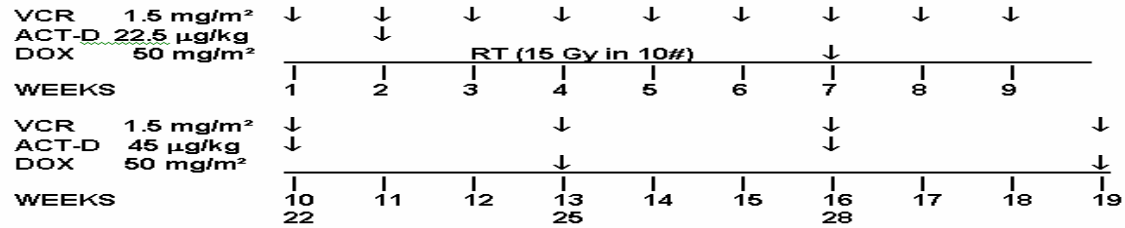
- *Regimen 1 (intensive VCR): Stage I, intermediate risk (excluding focal anaplasia).* → 10 weekly injections of vincristine 1.5 mg/m² as a single agent.



- *Regimen 2 (AV): Stage II, low and intermediate risk and stage I, focal anaplasia.* → 11 weekly injections of vincristine, then three weekly for 5 further doses, together with actinomycin D every three weeks starting at week 2 for a total of 9 doses. Total duration of treatment: 6 months.



- *Regimen 3 (sequential AVD): Stage III intermediate risk. Low risk: no radiotherapy.* → "*sequential AVD*", consisting of 10 weekly doses of vincristine, followed by 6 further doses at three-weekly intervals; actinomycin D 22.5 ug/Kg at week 2 just prior to radiotherapy and then at 45 ug/Kg at week 10, 16, 22, 28; doxorubicin 50 mg/m² at six weekly intervals alternating with actinomycin D, starting at week 7. Total duration of treatment: 28 weeks. Total doxorubicin = 200 mg/m^2.



- *Regimen 4 (VCCD): Stage IV (if there is no CR after 6 weeks of AVD), low and intermediate risk.* Patients with metastases, who are primarily operated, receive primarily the preoperative chemotherapy as for stage IV. The further treatment depends on the response of the metastatic disease. If CR is achieved, these patients will be treated according to those patients with preoperative chemotherapy (AVD). In case of Non-CR they will receive VCCD.

II.3.14 Chemotherapy: Drugs and Dosage

No dose of actinomycin D or doxorubicin and no course of carboplatin, cyclophosphamide or etoposide should be initiated if the absolute neutrophil count is <1.000 /mm^3 or the platelet count is < 100.000 mm^3.

- **Actinomycin D (Lyovac®, Cosmegen®)**
  Sharp and Dohme
  Vials 0.5 mg lyophilized powder
  45 μg/kg/once course for children who weigh 12 kg or more.
  30 μg/kg/once course for children who weigh less than 12 kg.
  No single dose should exceed 2000 μg.

- **Vincristine sulfate (Oncovin®)**
  Eli Lilly
  Vials 1 mg/1 ml, 2 mg/2 ml, 5 mg/5 ml solution.
  1.5 mg /m²/once weekly for children who weigh 12 kg or more.
  1 mg/m²/once weekly for children who weigh less than 12 kg.
  No single dose should exceed 2 mg.

- **Doxorubicin**
  Pharmacia & Upjohn SA
  Vials 10 mg, 20mg, 50 mg, 100 mg or 150 mg lyophilized powder
  Vials 10 mg/5 ml, 20 mg/10 ml, 50 mg/25 ml or 200 mg/100 ml
  50 mg/m^2/once/course for children who weigh 12 kg or more.
  33 mg/m²/once/course for children who weigh less than 12 kg.
  Total cumulative dose given should not exceed 3.0 mg/m^2

- **Etoposid (VP-16), (Etoposide®)**
  Pierre Fabre Oncologie
  Vials of 5 ml containing 20 mg/ ml.
  OR
- **Etoposid phosphate (Etopophos®)**
  Bristol-Myers Squib
  Vials of 100 mg lyophilized powder.
  150 mg/m^2/daily for 3 consecutive days for children who weigh 12 kg or more.
  100 mg/m²/daily for 3 consecutive days for children who weigh less than 12 kg.
  Total cumulative dose given should not exceed 2700 mg/m^2.

- **Carboplatin (Paraplatin®)**
  Bristol-Myers Squib
  Vials of 50 mg/5 ml, 150 mg/15 ml and 450 mg/45 ml solution.
  200 mg/m²/daily for 3 consecutive days for children who weigh 12 kg or more.
  2/3 dose (mg/m²) daily for 3 consecutive days for children who weigh less than 12 kg.
  Nephrotoxicity is of importance at high doses and in patients with prior renal dysfunction.
  Precautions: reduction of the dose in proportion to creatinine clearance.
  Total cumulative dose given should not exceed 3600 mg/m^2.

- **Cyclophosphamide (Endoxan ®)**
  Asta
  Vials of 100 mg, 500 mg or 1 g lyophilized powder
  450 mg/m^2/daily for 3 consecutive days for children who weigh 12 kg or more.
  300 mg/m^2/daily for 3 consecutive days for children who weigh less than 12 kg.
  Total cumulative dose given should not exceed 8100 mg/m^2.

- **Mesna (Uromitexan®)**
  Asta
  Vials of 400 mg/4 ml, 1 gr/10 ml or 5 gr/50 ml solution.

- **G-CSF (Lenograstime, Filgrastime) (Neupogen®)**
  Amgen-Roche
  Vials of 300 mcg/1 ml or 480 mcg/1,6 ml of rH G-CSF solution
  5 μg/kg/daily 48 hours after the last dose of chemotherapy and given until
  ANC> 10.000 and past the nadir of myelo suppression or a minimum of 1 week.
  Should be stopped for 48 hours before restarting chemotherapy.

II.3.15 Administration

Drugs should be preserved and reconstituted according to the instructions given by the manufacturer. Adequate hydration should be given to all patients receiving chemotherapy, *especially those less than 1 year of age, to avoid veno-occlusive disease.*

a) ***Actinomycin D, Vincristine****.* Are usually given directly into the vein without the use of an infusion, care should be taken to establish a good veno-puncture as, if extravasation occurs during injection, severe pain and tissue necrosis will occur. A method often used is to test the correct position of the needle in the peripheral vein by injections of physiological saline and by flushing the needle afterwards in the same way.

b) ***Doxorubicin****.* In order to minimise cardiac toxicity, it is generally agreed that doxorubicin should be given as an intravenous infusion over 24 hours/ 200 ml normal saline. There is not yet sufficient evidence that prolonging infusion beyond six hours is advantageous in further reducing toxicity, although it is possible that this will be the case. It is recommended therefore that each dose of doxorubicin be given by a slow intravenous infusion over a period of not less than 6 hours, and no longer than 48 hours, according to local policy. Regular monitoring with echocardiography is recommended during and after therapy. Central venous access is mandatory.

c) ***Etoposide*** (VP-16) (Vepesid®). Etoposide is made up in normal saline and should be infused over a period of 2-4 hours depending on volume. Concentrations > 0.5 mg/ml may precipitate before administration. The preferable concentration of this drug is 0,4 mg/ml. If more than 100 mg is to be given, this means that the total amount of infusion fluid is more than 250 ml. If given in one hour one should pay attention to this. No additional hydration is required for etoposide itself.

d) ***Carboplatin*** (Paraplatin®). The drug is dissolved in 250 ml glucose 5% solution and given over 4 hours.

e) ***Cyclophosphamide*** (Endoxan®). Must be accompanied by mesna (Uromitexan) to prevent bladder toxicity. Hydration should precede the infusion and continue during 12 hours, paying attention to the diuresis: patients should be asked to void at least every 2 hours during the 12 hours period immediately following a dose of cyclophosphamide. The drug is reconstituted with sterile water to a concentration of 20 mg/ml and should be administered as an IV infusion over 60 minutes.

f) ***Mesna*** (Uromitexan®). 30 minutes before the cyclophosphamide administration 20% of the cyclophosphamide dose in mg of Mesna. Thereafter every 4 hours again 20% of the cyclophosphamide dose until 24 hours after the last cyclophosphamid administration.

g) ***G-CSF****.* Is administered once daily subcutaneously or i.v. without dilution.

II.3.15.1 Dose modifications

- ***Adjustment of dose to body weight.*** Children with a body weight of less than 12 kg will have a dose reduction of 2/3 of the original dose mainly to prevent the risk of veno-occlusive disease.
- ***Radiation.*** If liver or large fields such as the entire abdomen, the entire thorax or both are irradiated, doses of actinomycin D should be reduced <u>for all patients</u> to 2/3 of the recommended dose during radiation and the first following course.
- ***Toxicity***

  1. **Hematological toxicity**. Hemoglobin level, WBC and platelet counts should be performed before each course of chemotherapy.

     Neutropenia: Absolute neutrophil count (ANC) has to be above 1000/mm^3 to start a course with actinomycin D or doxorubicin. Vincristine when given alone

may be continued without taking the ANC into account if the patient is clinically well.

Thrombocytopenia: Platelet count has to be > 150.000/mm^3 to start a course. The course in progress should be interrupted if the platelet count falls below 50.000/mm^3 and in case of such a sudden fall, the patient should be viewed with caution and the count repeated daily. Platelets transfusion is indicated only in case of haemorrhages.

Anemia alone: Should be treated by transfusion if necessary (Hb 7 g/l) but is not an indication to modify the treatment schedule.

If a course of treatment results in a nadir WBC count below 1500/mm^3 or in a nadir ANC below 1000/mm^3, associated with mucositis and/or fever or in a nadir platelet count below 50.000, associated with marked enlargement of the liver and or haemorrhages:

The doses on the next course should be reduced to 2/3 and if the next course of chemotherapy is well tolerated full doses will be tried again in subsequent ones.

2. **Isolated gastrointestinal complications**

Vomiting: Particularly occurs for a few hours after the injection of actinomycin D or doxorubicin. It usually can be treated symptomatically and rarely requires treatment modifications.

Diarrhoea: With or without vomiting particularly occurs after irradiation of the whole abdomen of young children. This may require the treatment to be withheld for a few days and sometimes irradiation has to be abandoned. Antispasmodics, intestinal antiseptics and intravenous fluids have to be given as required.

Constipation: Is common with vincristine. One has to see that loose stools are produced. The drugs should be omitted in case of paralytic ileus and restarted at a 50% dose.

3. **Hepatic complications**. May occur at the time of treating nephroblastoma of the right kidney and irradiation of the whole abdomen associated with actinomycin D or doxorubicin. They may be related too to actinomycin D alone. Patients with signs of liver dysfunction should be monitored carefully. Patients with severe liver diseases (VOD) should not be given actinomycin D until the main abnormalities have returned to normal and the dose should be reduced to 2/3 for the first following course. If the symptoms reappear during actinomycin D treatment, this drug should be permanently withdrawn. Vincristine may also enhance hepatopathy. If there are problems in interpreting or applying the protocol in children with hepatic disease, the secretariat should be contacted in writing and they will send the necessary instructions.

4. **Contamination or infection with varicella herpes**. Patients who develop varicella or herpes should receive Aciclovir and chemotherapy should not be restarted until one week after the resolution of the rash. It is advised to postpone all courses of actinomycin D and doxorubicin in case of contamination in non-immunized patients three weeks after a known exposure. If a course is postponed for that reason, one should aim at getting the patient back on the original schedule.

5. **Cardiac toxicity.** No generally accepted guidelines are available on which dose modification of doxorubicin can be based. There is some evidence that by the use of a 24 hour continuous infusion schedule, the risk of long term cardio-toxicity may be reduced. Monitoring with echocardiography should be done before the first administration and after every 100 mg/m^2 cumulative dose. Dose modification must be considered if fractional shortening falls below 28% or a reduction of > 10% is

seen between two consecutive administrations.Cardiac toxicity is more prone to occur in a patient who has received thoracic radiotherapy.

6. **Neurological toxicity**. Muscular weakness and hyporeflexia are the main side effects of vincristine. Jaw pain, pain on swallowing and hoarseness may occur. In case of peripheral nerve palsies, feat drop, and severe neuritis one or two injection of vincristine should be omitted and the next dose decreased to 2/3.
7. **Bladder and renal toxicity**. Cyclophosphamide can cause haemorrhagic cystitis if the details for its prescription are not met. For haemorrhagic cystitis, the treatment is only stopped if haematuria is macroscopic and repetitive. In the case of haemorrhagic cystitis: to increase diuresis a diuretic may be added: furosemide (Lasix) (0,5 mg/kg) 2 and 6 hours after the injection. Mannitol is also used under these circumstances.

II.3.16 Major Intolerance during re-Operative Therapy

It is an indication to cease chemotherapy if during the pre-operative chemotherapy the following complications occur.

- Profound thrombocytopenia with or without haemorrhages associated with veno-occlusive disease: abdominal pain with diarrhoea, ascites, oedema, marked enlargement of the liver, oliguria, fever and jaundice or with cutaneous erythema with desquamation or pruritis.
- Severe neurological complications as intolerable paresthesias with paralysis, convulsion, coma or amaurosis.

II.3.17 Supportive Care

The physician can prescribe whatever he thinks appropriate for pain, vomiting, constipation, etc.

- ✓ **A diet** containing no lactose, saccharose and gluten has to be given as a prophylactic measure during the irradiation.
- ✓ **Pneumonitis prevention:** In patients receiving the high risk regimen and those who are treated with lung irradiation it is recommended to give **Trimethoprim**.
- ✓ **G-CSF** is indicated in patients treated according to high risk regimen.
- ✓ For patients treated according to the other regimens, supporting treatment with growth factors is permitted but not considered as essential.
- ✓ **Transfusion:** Full blood and platelet transfusions may be given by centers recommendations and/or clinical protocols.

II.4 Surgical Technique, Recommendations and Advice

The principles of nephrectomy for paediatric malignancy were established by Gross in 1993: a wide transverse transabdominal incision and transperitoneal approach with early ligation of the renal vessels (67, 102, and 113). Pre-operative chemotherapy as demonstrated by SIOP studies 2, 5, 6, 9 and 93-01 makes nephrectomy easier and less hazardous. Furthermore, metastases may disappear or become resectable, vascular extension may regress and partial nephrectomy may become possible (35, 37, 49, 52, 65, 74, 151, 153).

The present study deals with further risk-adaptation of treatment. A number of patients will receive less treatment. Thus surgical staging, quality of resection and accurate reporting are of the utmost importance.

- ***General Remarks***
  1. Please read this chapter and the surgical questionnaires prior to operation.
  2. Excision of Wilms' tumour is an elective procedure and therefore, should be done by the most experienced team available.
  3. An emergency may occur if the tumour ruptures or bleeds pre-operatively and conservative management is ineffective. In spite of these difficulties, it is usually possible to follow most of the protocol requirements.

II.4.1 Imaging

Abdominal ultrasonography, plain chest X-ray in two planes and 24 hrs urine collection to assess the catecholamines excretion, kidney function and the presence of haematuria and tumour cells, are sufficient to establish the correct diagnosis in the majority of cases.

The imaging at presentation and after chemotherapy should be carefully assessed prior to operation.

Ultrasonography (US), with high quality Doppler (color or power) and computed tomography (CT) are of most value to the surgeon. The CT makes excretion urography (EU) unnecessary, but not the Doppler US. Magnetic resonance imaging (MRI) isvery effective but in most cases is unnecessary. An ambiguous sonogram should always be supplemented with CT or MRI. The surgeon must know the extent of the tumour, its location within the kidney, and its relation to the central vessels, diaphragm, liver, pancreas, spleen and adrenal glands **before operation**. Enlarged intra-abdominal (mainly para-aortic) lymph nodes, intra-abdominal metastases (mainly hepatic) and thrombus in the renal vein or vena cava should be sought. Chest CT is adviced if there is any doubt about the X-ray and if metastasectomy is planned.

II.4.2 The operation

- ***Access.*** Long transverse transabdominal incision or thoracoabdominal approach.
- ***Inspection of the Abdominal Cavity.*** Abdominal cavity should always be inspected prior to tumour removal. Metastases in the liver, lymph nodes and peritoneum should be searched for. Since SIOP 6 and 9 studies have shown the value of excision for both pulmonary and intra-abdominal metastases, every effort must be made to remove these completely (36, 53). Every lesion should be excised (if resectable) or biopsied (if unresectable) and its position marked. This includes lymph nodes, which should be sampled even if they appear normal (see below). Excised material must be sent to the pathologist in a separate container and its origin clearly indicated. Complete excision should be attempted even if the diagnosis of nephroblastoma is uncertain. Biopsy of the tumour should only be considered if it is inoperable because of the risk of spillage.

  Thorough inspection of the opposite retroperitoneal space is obligatory only if pre-operative imaging indicates bilateral localisation of the tumour. In other cases it rarely gives more information than good quality imaging. The operating surgeon should decide whether or not to do it in individual cases. Unequivocal stage V cases will be treated following "Stage V treatment suggestions".
- ***Nephrectomy.*** Early ligation of the renal vessels should be the aim and is possible in nearly every case. The renal artery should be ligated first in order to avoid swelling of the tumour with increase of its fragility and the possibility of dissemination via perforating perinephric veins. An extensive Kocher-manoeuvre of the duodenum is a convenient approach to the renal vessels for alarge tumour whether on the right or left. An approach via the peritoneum lateral to the colon is also acceptable. The technique of the approach should be indicated in the surgical questionnaire. If the tumour is very large and infiltrating and the primary ligation of renal vessels is difficult and considered too risky, the tumour is dissected from sourranding structures first, and vessels are ligated when possible. This

should be precisely described in the in the surgical questionnaire. The tumour should be removed together with adipose capsule and, if possible with all invaded surrounding structures. Heroic and mutilating resections such as pancreatectomy are not recommended as these tumours are both chemo- and radiosensitive (67, 102, 113).

- ***Renal vein, Vena, Vena cava.*** Although intravascular extension of the tumour is usually apparent on the pre-operative imaging, the vena cava and renal vein should be carefully examined during the operation. If thrombus is found, it should be removed. A short thrombus in the renal vein may be resected together with the vein. A thrombus extending to the infra-hepatic vena cava should be removed through a vena cavotomy, after occluding the contra lateral renal vein and cava above and below the thrombus. The thrombus should be removed and the venotomy closed. A longer thrombus, (intra-hepatic, supra-hepatic, or right atrial), may require the assistance of a vascular or cardiac surgeon and cardiopulmonary by-pass (33, 102, 113, 149). In cases with very extensive infiltration of the vena caval wall, the risks and benefits of surgery should be reconsidered. Even with extensive vascular surgery it may be impossible to achieve complete excision and radiotherapy may be a better option. The SIOP 9 study showed that not all such cases are lost (49).
- ***Adrenal Gland.*** The adrenal gland can be left in situ if a safe resection margin between the tumour and the gland can be guaranteed.
- ***Ureter.*** The ureter should be resected as close to the bladder as possible.
- ***Lymph Nodes.*** The NWTS 1 trial showed that 8/224 (3.6%) of lymph nodes that were declared negative by the surgeon showed metastases on histological examination and 25/64 (39.1%) of cases that were declared positive, did not.

  → **The tumour must not be upstaged if there is no histological confirmation of lymph node involvement.**

  Recent studies revealed a higher incidence of local recurrence in patients enrolled in NWTS-4 in whom biopsy of lymph nodes was not performed. This suggested that inadequate staging led to under-treatment of local disease in these children (130).

  → **Sampling and histological examination of lymph nodes is imperative for accurate staging and subsequent treatment.**

  Hilar and para-aortic lymph nodes at the origin of the renal artery (regional nodes) and nodes below or above this level (extra regional nodes) should be sampled even if not suspicious. Involved or suspicious lymph nodes must be excised without rupture. They must be carefully labelled and sent to the pathologist separately with an accurate description of their position and character.

  → **The above information affects staging, treatment and therefore outcome, radical lymph node dissection does not enhance survival and therefore is not part of the surgical therapy** (84, 92).

II.4.2.1 Stage IV Treatment Recommendations

1. As demonstrated by previous studies, lung metastases should be excised if possible (35, 36, 53). Operation should be performed as soon after nephrectomy as the patient's condition permits, typically within 14 days. Bilateral resectable lung metastases should be excised either via two thoracotomies or one sternotomy depending on surgical choice and anatomy. Wedge resections can frequently be radical. If wedge resection will not achieve complete excision then segmentectomy or lobectomy is acceptable. Pneumonectomy is not justified.
2. Experience from SIOP 6 and 9 justifies a similar approach for extra pulmonary metastases, especially for the second most frequent – in the liver (53). Wedge resection should also be appropriate in these cases. If liver involvement would require excision of more than one segment surgical treatment is probably

inappropriate, and should be postponed after further chemotherapy reduces its extention (53). Metastases outside lung or liver should be excised completely provided the operation can be done without mutilation, or loss of vital organs (35, 36, 53).

3. Complete excision of metastases is extremely important as it removes the need for irradiation. It is rarely successful when metastases do not respond to chemotherapy.
4. The sampling of hilar and para-aortic lymph nodes is just as important in patients with metastases.

II.4.2.2 Partial nephrectomy

Partial nephrectomy may assure local control in Wilms' tumour (28, 48, 58, 74, 79, 108, 109). Most reports deal with bilateral tumours in which this approach is the management of choice. Unilateral cases may also benefit from partial nephrectomy, but the advantages and risks have to be precisely evaluated for each individual case (28, 48, 58, 74, 79, 108, 109). Contra lateral urological and nephrological disorders and genetic syndromes of an increased risk of Wilms' rather than a risk of hyper perfusion nephropathy in the remaining kidney are important criteria when this option is considered (69).

We do not recommend partial nephrectomy in a classical unilateral nephroblastoma which is not related with the above disorders.

We would like to collect all data on patients subjected to partial nephrectomy provided that the contraindications listed below are respected.

- ***Contraindications for partial nephrectomy:***
  - preoperative tumour rupture or biopsy
  - tumour infiltrating extra renal structures
  - intra-abdominal metastases or lymph nodes seen on preoperative imaging
  - thrombus in the renal vein or vena cava
  - tumour involving more than 1/3 of the kidney (at least 50% of renal tissue should be spared after the tumour resection with a margin of healthy tissue, to give any worthwhile protection against hyper perfusion).
  - multifocal tumour,
  - central location,
  - involvement of calyces
  - haematuria
  - little experience in partial nephrectomy.

- ***Remarks:***
  - A significant reduction of tumour volume after the preoperative chemotherapy suggests better chance for successful partial nephrectomy.
  - Resection must be performed with the margin of healthy renal tissue; enucleation is not adequate local treatment.
  - Intra-operative ultrasound scanning is very useful in defining the intrarenal tumour extent.
  - Following partial nephrectomy the kidney should be assessed with Doppler sonography (or IVP) two days after surgery. The contribution of the spared renal tissue in the total urinary excretion should be assessed scintigraphically 6 months later. Further long-term functional f ollow-up is mandatory.
  - Patients with stage I anaplastic tumours after pre-operative chemotherapy have a higher risk of relapse than those after immediate nephrectomy (159). Nephrogenic

rests, in the renal parenchyma of the partial nephrectomy specimen, may give rise to metachronous nephroblastoma in the residual kidney. These patients should be followed very carefully after partial nephrectomy with ultrasonography performed monthly for at least six months. Subsequently the standard follow-up is continued.

- The decision for partial nephrectomy should be taken by all members of treating team and finally approved by the surgeon at operation.

II.4.2.3 Surgery related complications

A joint NWTS-SIOP Study on surgery related complications is in progress. Previous SIOP reports demonstrated marked reduction surgery-related complications when pre-operative chemotherapy was compared with primary nephrectomy.

The aim of the study is to verify the retrospective results of the NWTS-3 (19.8% surgery-related complications rate after primary nephrectomy ignoring tumour rupture) and the SIOP-9 (8% surgery-related complication rate after post-chemotherapy nephrectomy including tumour rupture) in the prospective way (52, 124). The forms and rules do not change: the "nephrectomy-related complications check list" should be consulted twice: at operation and 1 year later. Completed data sheets should be sent to the SIOP Nephroblastoma Trial and Study Office.

II.4.3 Closing Remarks

The standard metal clips, however useful for many reasons, should be avoided if CT or MRI is planned. Please use titanium clips which do not interference with either CT or MRI).

All suspicious structures should be biopsied or resected, marked, described precisely and sent to the pathologist in separate containers.

At nephrectomy, areas of dubious complete excision should be marked and described precisely on both surgical and pathology forms. A copy of the complete surgical report should accompany the surgical questionnaire.

Please complete the drawing enclosed with the questionnaire for every surgical procedure, and add comments after review with your pathologist. This should be included with the completed forms.

Please fill in one "metastatectomy questionnaire" for each metastatectomy you performed even if it was performed during nephrectomy. One copy of the complete metastatectomy report should accompany the questionnaire. Since nephrectomy and metastatectomy may be performed in different hospitals, the responsible paediatric oncologist should ensure that both operating surgeons complete the relevant questionnaire.

II.5 Radiotherapy

II.5.1 Indications, Aims, Equipment and Target Volume

- ***Indications for post-operative flank RT:***
  - Histologically intermediate risk, stage III (nodes postive N+, residual disease left after surgery, tumour rupture)
  - High risk, stage II and stage III
  - Stage IV and stage V according to local stage
- ***Indications for post-operative whole abdominal RT:***
  - Whole abdominal RT is indicated for DIFFUSE intra-abdominal tumour or GROSS pre-operative or peri-operative rupture.
  - Abdominal / flank RT will start as soon as possible within 2-3 weeks after abdominal surgery.

- ***Indications for pulmonary RT:***
  - Residual tumour tisssue in the lungs is visible on a chest X-ray or CT scan after the commencement of pre-operative chemotherapy, and that this residual tumour is not completely excised, or if post-operative chemotherapy according to the high risk protocol doesn't lead to a complete remission.
  - RT should not be given if a CR is achieved following a six week duration of chemotherapy.

- **Aims Of Radiotherapy**
  - To achieve control of abdominal disease in patients who have significant risk of intra-abdominal relapse.
  - To increase the control of pulmonary metastases in patients who do not achieve a complete remission.

- **Equipment.** Modality: photons from a linear accelerator. If not available one may use Cobalt-60. Energy usually 4-6 MV.

- **Target Volume.** Target Volumes are defined according to ICRU 50 and ICRU 62 guidelines (81, 82).

II.5.2 Localisation of primary tumour and kidney for flank/abdominal Radiotherapy

For RT planning the tumour extent should be localised according to the surgical and histopathological reports, and pre-operative ultrasound and pre-operative contrast-enhanced CT scan if available. (If not available conventional urography, frontal X-ray film with tumour lineated by a metal thread pre- and intra-operatively can also be used.)

The boundaries of the tumour and kidney during surgery must be marked with clips and in the case of areas suspicious of incompletely resected disease these should be marked with clips (material which does not interfere with CT or MR imaging) as well.

Marking the boundaries of the tumour/kidney is probably the most important way of delineating the tumour and its extension.

A margin of two cm should be taken superior, lateral and inferior of these clips. The medial border always encompass the full width of the vertebral bodies.

In the case of pre-operative or intra-operative rupture the anatomic location and the intra-abdominal space (intra/retro-peritoneal) should be clearly indicated in the surgical note and drawing. Infiltration into the peri-renal fat, involved lymph nodes, macroscopic incomplete resection, microscopic or macroscopic ruptures have to be stated clearly.

- **Indications for hepatic RT**: Liver metastases which do not respond completely to chemotherapy and which cannot be completely resected with negative margins.
- **Indications for RT to other metastatic sites**: Haematogenous metastases brain (whole brain RT) and/or bone metastases (focal RT) at diagnosis.
- **Simulation**: All patients will undergo a simulation procedure with a conventional simulator or CT-simulator. All patients will be treated in the supine position. Customized blocks are drawn on the simulator films and will be checked on the simulator. All critical organs will be blocked if this is possible.

II.5.2.1 Clinical Target Volume (CTV)

- **Flank RT.** CTV: This encompasses the extent of post-chemotherapy and pre-operative macroscopic tumour and the kidney according to the surgical and histopathological

reports and according to the extent on CT-scan/ ultrasonography. The margin for CTV is 2 cm. The **treated volume** should extend across the midline to achieve homogeneous irradiation of the full width of the vertebral bodies.

- **Boosts for residual macroscopic disease.** CTV: This should encompass the extent of macroscopic residual disease after surgery with a margin of 2 cm.
- **Whole abdominal RT.** CTV: This includes the entire abdominal contents and peritoneum extending from the dome of the diaphragm to the pelvic floor (lower border of obturator foramen).
- **Pulmonary RT.** CTV: This encompasses both lungs including the apices and costo-diaphragmatic recesses. If also abdominal radiotherapy has to be given, both fields should be matched in order to avoid any gap or overlap.
- **Liver RT.** CTV: This includes the extent of incompletely resected tumour with a margin of 2 cm.
- **RT for brain metastases.** CTV: the whole brain is treated.
- **RT for haematogenous metastases to bone.** CTV: For bone metastases the entire bone need not be treated. The field includes the obvious disease visible on imaging examination, with a margin of not less than 3 cm in any direction.

II.5.2.2 Planning target Volume (PTV)

Margins for PTV will be influenced by individual departemental policy. In general the margins that will be applied will be as follows:

- Internal margin: 1-2 cm for breathing movements.
- Set-up margin: for variations in the daily set-ups 0.5- 1 cm.
- These margins may need to be reduced in case of proximity of critical organs.

II.5.2.3 Treatment Dose

Prescription Point: the mid-plane of the central axis for parallel-opposed fields (ICRU 50 definition).

- **Flank RT:** Total dose is dependent on stage and pathology. Fraction dose is conditioned by the age of the child and the volume encompassed. Stage III low and intermediate risk: 14,4 Gy Boost to the macroscopic residual disease after surgery: 10.8 Gy (giving a total dose of 25.2 Gy). Stage II, stage III, high risk: 14,4 Gy Boost to the macroscopic residual disease after surgery: 10.8 Gy.
- **Whole abdominal RT:** The entire peritoneal cavity should be irradiated to a maximum of 20 Gy, with the consideration of a boost to a limited area (as for flank RT). Dose per fraction should be lowered to 1.5 Gy. A milk and gluten-free diet should be considered for the duration of the abdominal radiotherapy. In children under one year of age total dose should be reduced to 10-12 Gy.
- **Brain RT:** The whole brain is treated to a dose of 25.5 Gy. A small boost may be given (4.5 Gy).
- **Liver RT:** A dose of 20 Gy may be given to the area of R1 resection of metastases.
- **Bone RT:** For bone metastases the metastasis may be treated with a dose of 30 Gy.
- **Pulmonary RT:** For whole lung RT the total dose is 15 Gy for both lungs (with correction of tissue heterogeneity). The dose per fraction is 1.5 Gy deliverd within 10 treatment days. A boost of 10 Gy-15 Gy should be considered for areas of gross residual disease after surgery.

II.5.2.4 Time Dose considerations

- **Daily dose.** The dose per fraction will be decided by the treating radiation oncologist and will depend upon the age of the child and the volume encompassed.
- **Flank RT.** The dose per fraction is 1,8 Gy, but may be lowered when large volumes are treated (e.g. whole abdomen).
- **Total abdominal RT.** The dose per fraction is 1.5 Gy, but may belowered to 1.25 Gy in case of toxicity and very young children (< 2 years).
- **Whole lung RT.** The dose per fraction is 1.5 Gy (with homogeneity correction).
- **Brain RT:** The dose per fraction is 1.5 Gy.
- **Liver RT:** The dose per fraction is 1.5 Gy.
- **Bone metastases:** The dose per fraction is 3 Gy.
- **Number of fractions per day.** Daily fraction, five days per week, Monday-Friday.
- **Rests/ Interruptions.** Rests must kept to an absolute minimum. Interruptions to treatment machine service and public holidays must be avoided unless absolutely unavoidable.
- **Interruptions for myelotoxicity.** RT should be interrupted if the neutrophil count falls below 0.5 x 10^9/L and should not be resumed until the count is at least 1.0 x 10^9/L. RT should be interrupted if the platelet count falls below 25 x 10^9/L and should not be resumed until the count is at least 50 x 10^9/L. The haemoglobin level should be maintained at a minimum of 10 g/dl during RT with correction by transfusion if necessary. GCSF may be used in the case of the neutrophil count falling below 0.5, and continued until it is grater than 1.0.

⇨ **Dose Uniformity and reference Points (ICRU 50).** The dose variation within the targetvolume should not exceed - 5 % - + 7% of the prescribed dose.

II.5.3 Tretament Technique

Patients will generally be treated in the supine position.

- ***Normal Tissue Sparing***
  - **Critical organ dose.** Remaining kidney: The dose to the remaining kidney should not exceed 12 Gy. Liver: the dose to the whole liver should not exceed 20 Gy. A dose exceeding 20 Gy should not be received by more than half the liver. Lung: the whole lung dose should not receive more than 15 Gy in 1.5 Gy fractions (with correction for inhomogeneity). A dose exceeding 15 Gy should not be received by more than 25 % of the lung volume.
  - **Shielding** Joints: For pulmonary RT the shoulder joints should be shielded. For whole abdominal RT the hips should be shielded.

- ***Quality Assurance Documentation***

  Copies of the following quality assurance information should be sent on request to the national clinical trials office or to the SIOP secretariat along with copies of the radiotherapy trial forms:
  - Radiotherapy Data reporting forms
  - Diagnostic/Clinical Data
  - Simulator films (initial volume and boost)
  - Treatment machine verification films
  - Daily treatment chart
  - Copies of dose calculations

Examples for typical target volumes and radiation portals: stage II high risk, stage III (related to anatomical landmarks) Fig. 9a, b, c

Cranial border:
- left sided tumours:1-2 cm above the macroscopic tumour e.g. dome of diaphragm

- right sided tumours:if feasible 1-2 cm below the dome of diaphragm (sparing of liver)

Caudal border: 1-2 cm below the macroscopic tumour e.g. within the iliac fossa often including the iliac crest. Watch the position of the ovaries (homo- and contralateral)

Lateral border:including the abdominal wall

Medial border : depending on tumour extension: including the vertebral bodies watch the contralateral kidney

→ **Boost volume for macroscopic residual disease:** extent of residual macroscopic disease at surgery with a 1-2 cm safety margin.

Examples for typical target volumes and radiation portals: stage II high risk, stage III residual disease, minor rupture (continued)

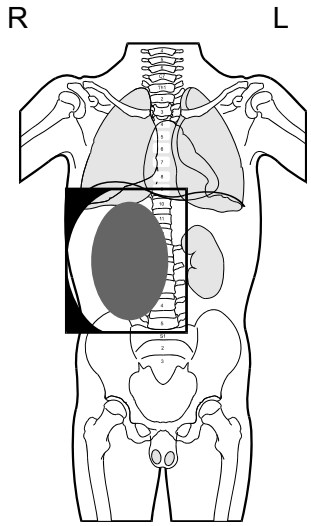


Figure 9a. Right sided tumour with microscopic residual disease and minor rupture (stage III). Radiation portal covering the tumour region including the vertebral column, the iliac crest and major parts of the right liver. The same type of radiation portal would apply for a nephroblastoma stage II, high grade.

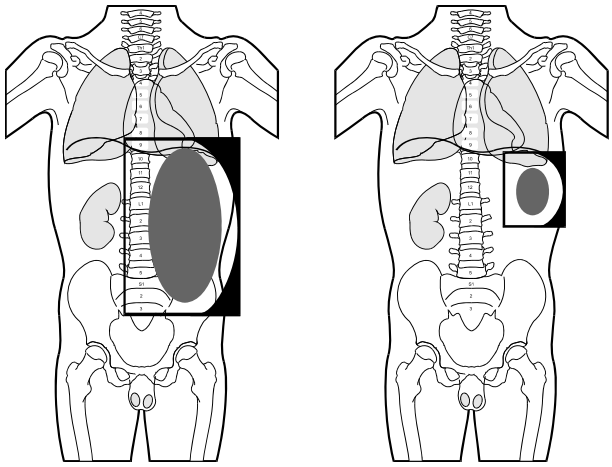


Figure 9b. Extensive left sided tumour from the dome of the diaphragm to the fossa iliaca with macroscopic residual disease at the splenic hilus (Stage III): little tumour shrinkage after pre-operative chemotherapy. Radiation portal including the major part of the left hemiabdomen with the vertebral column; boost portal including the left upper abdomen without the vertebral column.

Examples for typical target volumes and radiation portals: Stage III tumour thrombus vena cava inferior (continued)

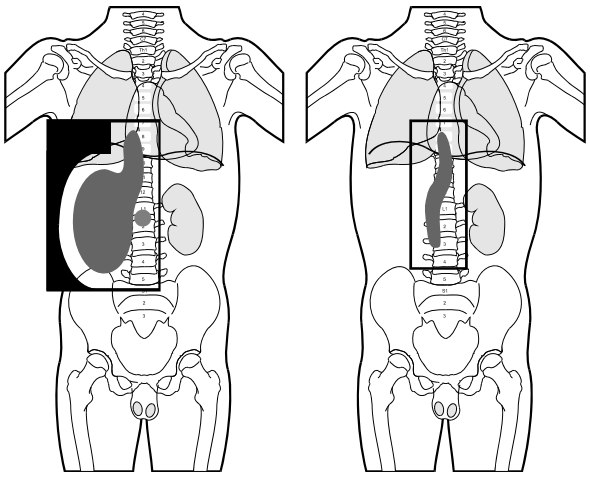


Figure 9c. Right sided tumour with paraaortic lymphnode metastases infiltrating the vena cava inferior up to the diaphragm and tumour thrombus up to the right atrium (Stage III): lymphnodes and tumour thrombus could not completely removed macroscopically by surgery. Radiation portal encompassing the tumour region, the paraaortic lymphnode chain, and the vena cava inferior including part of the right atrium. Boost portasl covering the area of the macroscopic residual disease: paraaortic lymphnode chain, vena cava inferoir, and part of the right atrium.

Stage III intermediate and high risk: (if lymphnode involvement under the level of the renal artery, same instruction): Target volume encompasses the whole paraaortic lymphnode chain including the homolateral pararenal lymphnodes and the macroscopic tumour extent at surgery plus a 1-2 cm safety margin. The safety margin may not be feasible towards the contralateral kidney. Examples for typical

Target volumes and radiation portals: Stage III (related to anatomical landmarks) Fig. 10, a,b

Cranial border:

- Left sided tumours: 1-2 cm above the macroscopic tumour e.g. dome of diafragm
- Right sided tumours: if feasible 1-2 cm below the dome of diafragm (sparing of liver)
- Lymphnode chain: upper plate of TH XII

Caudal border: 1-2 cm below the macroscopic tumour e.g. within the iliac fossa often including the iliac crest. Watch the position of the ovaries (homo-and contralateral)!

Lymphnode chain: Lower plate of L IV (lymphnode chain) but for preventing inhomogeneous dose to the bone, the border is often the elongation of the caudal border

Lateral border: including the abdominal wall

Medial border: including the tranverse process of the vertebral column. Boost volume for lymphnode chain in case of macroscopic residual disease in the lymphnodes (stage III):

Cranial and caudal border: see above

Homolateral border: including the tranverse processes of the vertebral column and the renal hilus

Contralateral border: including the transverse process of the vertebral column

Examples for typical Target Volumes and Radiation Portals: Stage III macroscopic residual disease (continued)

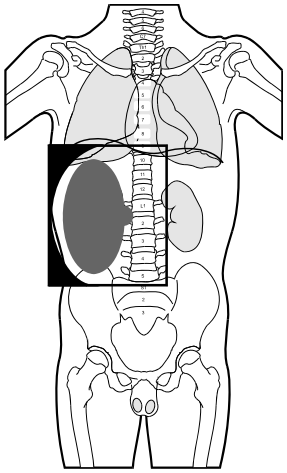


Figure 10a. Right sided tumour with one homolateral pararenal lymphnode involved and removed (stage III). Radiation portal covering the tumour region (including the right dome of diafragm and the iliac crest) and the whole paraaortic chain.

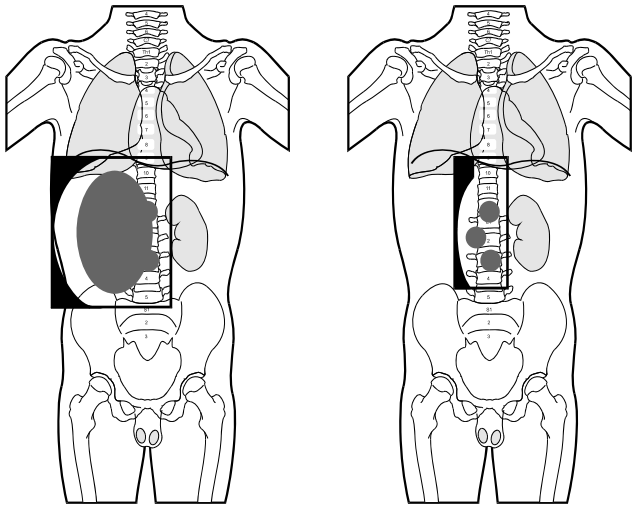


Figure 10b. Right sided tumour with several paraaortic lymphnodes involved (stage III) and suspicious macroscopic residual disease in the lymphnode chain at surgery (stage III macroscopic residual disease). Radiation portal covering the tumour and the paraaortic lymphnode region. Boost volume in case of macroscopic residual disease refined to the lymphnode chain including the homolateral renal hilus.

Examples for typical target volumes and radiation portals: Stage III major intraperitoneal rupture (continued)

Stage III (all histologies): major intraperitoneal rupture. Target volume encompasses the whole intraperitoneal cavity.

Examples for typical target volumes and radiation portals: stage III major intraperitoneal rupture (related tot anatomical landmarks) fig.11

Cranial border: including both domes of the diaphragm

Caudal border: upper part of the symphysis

Caudal and lateral border: line along the inguinal ligament (sparing the epiphyses of the femoral head)

Lateral border: including abdominal wall. Watch shielding the remaining kidney (max dose 12 Gy) watch dose at the testes

→ **Boost Volume for macroscopic residual disease**: extent of residual macroscopic diseas at surgery with a 1-2 cm safety margin.

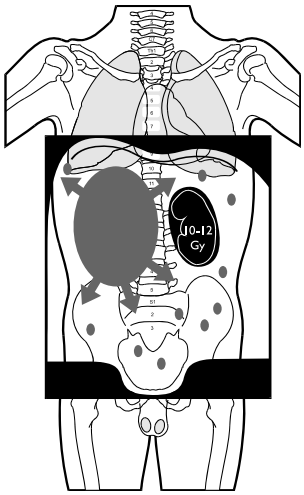


Figure 11. Massive intraperitoneal rupture during surgery as right sided tumour broke into many pieces and spread around the intraperitoneal cavity (stage III major rupture). Radiation portal covering the whole intraperitoneal cavity. No boost indicated as no detectable macroscopic residual disease was seen at surgery.

Examples for typical target volumes and radiation portals: stage III major retroperitoneal rupture (related and anatomical landmarks) fig. 12

Stage III (all histologies) major retroperitoneal rupture. Target volume encompasses the whole homolateral retroperitoneal space including the prevertebral space.

Cranial border: including the dome of the diaphragm

Caudal border: upper part of the symphysis

Caudal and homolateral border: line along the inguinalligament (sparing the epiphyses of the femoral head)

Homolateral border: including the abdominal wall

Contralateral border: including the vertebral bodies, line from edge of LV to symphysis (watch the location of the contralateral ovary! Watch the dose at the testes!).

→ **Boost volume for macroscopic residual disea**se: extent of residual macroscopic disease at surgery with a 1-2 cm safety margin.

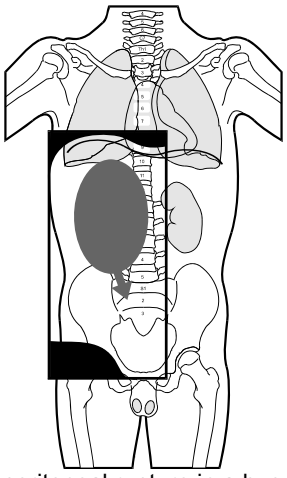


Figure 12. Extensive retroperitoneal peritoneal rupture in a huge tumour without contamination of the intraperitoneal cavety (stage III major retroperitoneal rupture). Radiation portal including the right retroperitoneal cavity and the retroperitoneal prevertrebral space. Boost is indicated if there is macroscopic disease left in the retroperitonal space during surgery.

➔ **Keep in mind:** When defining the margins of the radiation field the following well known fact has to be taken into account: geometric field margins by definition represent in most megavoltequipments the 50 %-isodose curve and do not represent the adaequate dosimetric target coverage.

II.5.4 Pulmonary Radiotherapy

II.5.4.1 Stage IV: Lung

Target volume encompasses both lungs including the costodiaphragmatic recesses.

If local abdominal radiotherapy has to be performed, pulmonary and abdominal targets are defined on the same film. If the targets overlap, a decision has to be taken related to target matching of the two adjoining radiation fiels. Special attention has to be paid tot radiation related morbidity when treating a larger volume.

Examples for typical target volumes and radiation portals: stage IV lung (related to anatomical landmarks) Fig. 5

Cranial border: including the top of the lung (some cm above the clavicle)

Cranial and lateral border: including the lung, shielding the shoulder region

Caudal border: including the bottom of the costodiaphragmatic recesses: e.g. 2-4 cm below the radiologically visible diaphragm, depending much on the phase of respiration which is to be sen al lateral recesses or on tranverse fluoroscopy

Lateral borders: including the thoracic walls

→ **Boost volume:** 5-10 Gy to tumour remnants visible at the start of radiotherapy. If very widespread, 5 Gy to the whole lung (up to 20 Gy). In very young children, protect as much lung tissue as possible.

Examples for typical target volumes and radiation portals: stage IV lung (continued)

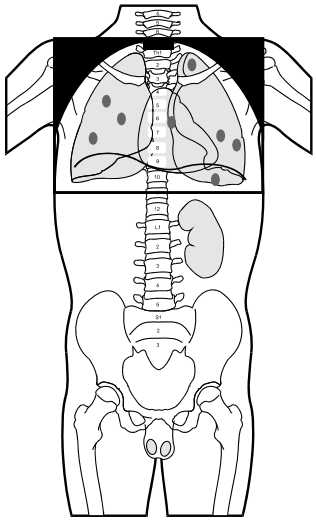


Figure 13. Pulmonary metastases at diagnosis (stage IV lung) with residual inoperable disease in the left and central right lung after pre-operative chemotherapy. Indication for pulmonary radiotherapy if there is disease after post-operative aggressive chemotherapy. Radiation portal including both lungs with its recesses. **Remember air-correction when calculating dose.** Lateral view needed to calculate dimension of lung tissue.

II.5.5 Organs at Risk

II.5.5.1 Bone and soft tissue

It is not clear to what degree a radiation dose of 15 Gy in young children will impair bone and soft tissue growth. It can be assumed that- if there will be an impairment- this will only be small and of no significant clinical relevance. The amount of impairment is certainly larger after a radiation dose of 30 Gy.

The whole vertebral column should always be included within the radiation portal in order to avoid dose inhomogeneity which is known to produce scoliosis. Nevertheless, the radiation portal should not include major parts of the contralateral kidney (fig. 9, 10)

The iliac crest contains the apophysis from which the growth of the iliac bone mainly takes place. In order to avoid asymmetric iliac bone growth radiation dose at this apophyseal line should not be more than 15 Gy (fig. 9, 10)

The epiphyseal lines of the acetabulum cannot be saved, if the whole intraperitoneal cavity is to be adequately irradiated (" abdominal bath") (fig.11, 12)

The femoral head should not be included in the treatment volume as it does not belong tot the target volume and epiphyseal slipping is a possible consequence after radiatherapy in young children (fig 11, 12)

The shoulder is not to be included within the treatment volume when pulmonary radiotherapy is indicated (fig. 13).

Due to technical reasons there is little chance to get soft tissue out of the treatment volume. The skin is spared by the build-up effect of megavolt beams. Sparing of the underlying soft tissue increases with megavoltage energy.

- **Liver.** Radiation tolerance of the liver depends on total dose and volume irradiated. A radiation dose of 15 to 20 Gy to the whole liver does not by itself produce severe side effects and is indicated in whole abdominal irradiation (15 Gy) and may be advisable in some extensive right sided tumours. If a boost volume is indicated in the upper right abdomen at least one fourth of the liver should be shielded after 20 Gy. If less than half of the liver is within the treatment volume no special shielding is necessary.

  If *veno-occlusive disease (VOD)* happened during chemotherapy, the radiation tolerance of the liver might be reduced. Special attention should be paid to further liver shielding.

- **Gastrointestinal tract.** Because of the radiosensitivity of the rapidly proliferating mucosa sparing from the irradiation volume is advisable but only possible by adequately tailoring the treatment portal.

- **Kidney.** Does to the remaining kidney is not to exceed 12 Gy. Irradiation of the remaining kidney up to 12 Gy is indicated in total abdominal radiotherapy and is some cases of stage V tumours. Radiation dose to the contralateral kidney in radiotherapy of the prevertebral space due to the penumbra at the field margin and scattered radiation usually does not exceed 10 to 20 % of the radiation dose at the reference point. It may be somewhat higher in medial parts in the remaining kiudney lying close to the vertebral column.

II.5.5.2 Reproductive Organs

- **Ovary.** At least one ovary should not receive a radiation dose (from scattered radiation, beneath a shielding block) of more than 10- 15 % of the dose at the reference point (15 Gy) (Fig. 9, 10, 12). As the necessary distance between the field margin and the location of the ovary to achieve this dose can be estimated before treatment performance (in using e.g. 10 MV photoins the distance should be more than 2 cm), much attention should be paid to adequate localization in this regard. If the target dose is 30 Gy the ovarian dose should not exceed 5-10% of the reference dose. Only in total abdominal radiotherapy both ovaries are irradiated up to 15 Gy.

- **Testes.** Radiation dose to the testes from scattered radiation should be clearly below 5 % of the radiation dose at the reference point (15 Gy). Special attention is necessary in total abdominal radiotherapy because of the close relationship between the caudal border and the position of the testes particularly in small boys (fig. 11, 12).

- **Mammarian Bud.** The mammarian bud as known to be very radiosensitive even in low dose radiotherapy should be spared from radiotherapy whenever possible. Special attention has to be paid when treating tumours in the upper abdomen and including the dome of the diaphram (fig. 9b, 11, 12). In radiotherapy of both lungs some sparing of the mammarian bud may only be achieved by the build-up-effect in high megavoltage beams (fig.13).

II.5.6 Technical and Physical Treatment Planning and Performance

Treatment planning is based on adequate tumour localization and target definition. It includes treatment simulation at a dedicated treatment simulator, production of individual, focussed shielding blocks, and calculation of dose.

Computed tomography within the planning procedure and computed assisted calculation of dose distribution based on transverse CT is recommended.

The most often chosen field arrangement are two parallel equally weighted opposed fields (from anterior and posterior)

II.5.6.1 Treatment simulation

At the treatment simulator the borders of the portals are precisely and reproducibly defined by fluoroscpic imaging, documented on a simulation film (X-ray-film) and drawn on the skin of the child. The child is in the same position (usually supine) as during the following treatment.

Tumour extension, target volume and shielding blocks are delineated on the simultion film based on the surgical and histopathological report (drawing) and pre-operative X-ray film and sectional imaging (CT). The position of the contralateral kidney is visualized on the simulation film by intravenous contrast medium.

In case of a CT assisted treatment planning tumour, target volume, and organs at risk are delineated on one or several CT slices taken with the child in treatment position.

- **Shielding blocks.** The kidneyblock should preferably placed in the posterior field only. The production is individual, focussed shielding blocks is based on the drawing on the simulation film and carried out by hands or computed assisted. The thickness of the blocks is dependent on the atomic number of the shielding material and the beam energy and should be at least 5 h.v.l. thick. Radiation dose below the shielding block should preferably be below 10 % and should not exceed 15 % of the dose at the reference point. When shielding the ovaries by blocking, thicker blocks, 6 h.v.l., are advised.

II.5.6.2 Calculation and Reporing of Dose in the Target and Organs at Risk

- **Target volume dose.** Target dose is calculated and reported according to the ICRU criteria. This reference point is in a central part of the target volume. For nephroblastoma treatment the reference point of target dose is specified as follows: for parallel opposed equally weighted beams (most usual) on the central axis midway between the beam entrances; for parallel opposed unequally weighted beams on the central axis at the centre of the target area; for any other arrangement of intersecting beams at the intersection of the central axis of the beams.

  Dose inhomogeneity within the target volume should be in ± 5 % of the dose at the reference point and should not exceed ± 10 %. CT based computed dose calculations have to follow the same rules for target dose specification. In pulmonary radiotherapy the dose at the reference point (central beam midway in the mediastinum) has to be corrected taking account the minor radiation absorption in the air filled lungs which represent the target volume. It results in a reduction of dose at the reference point about 10-15 % in order to arrive at the prescribed dose in the lung.

The distance at which the 90%-isidose is reached from the 50%-isodose at the geometric field margin towards the centre of the portals depends on the beam sharpness. Megavoltage equipment, beam quality and energy, source size and source surface distance, field size, depth of reference point are all factors influencing the beam sharpness.

- **Dose in organs at risk.** Dose in organs at risk is calculated and reported for each organ separately. It is recommended to add the (estimated) volume of the organ irradiated tot the reported dose. Typical organs at risk in nephroblastoma treatment are the vertebral column, iliac bone, contralateral kidney, soft tissue of the irradiated flank, liver, ovaries, testes.
- **Treatment Performance.** Patients are treated on megavoltage equipment with modern technical refinement (e.g. gantry rotation, isocentre, beam collimation). Modern linear accelerators are very suitable machines for these treatments. Cobalt 60 units (SSD at least > 80 cm) may also be used regarding the physical and technical properties. Patients are treated in supine position through anterior and posterior portals (by gantry rotation) which are equally weighted. Both fields are treated every day. Divergent shielding blocks are positioned on the tray holder.

  Verification films are taken at the megavoltage beam before the first treatment and at regular intervals at least once a week.

  A photograph is taken with the contours of the treatment field and the shielding blocks drawn on the skin of the child. For megavoltage photon energy above 10 MV, bolus is needed because of the low dose in the first 1-2 cm
- **Side effects.** Significant acute hematologic side effects (neutropenia, thrombocytopenia) are observed when irradiating extensive volumes including a large ammount of bone marrow together with chemotherapeutic agents that lead to significant hematological toxicity by itself (actinomycin D, epirubicin, carboplatin). Therefore the dose of these chemotherapeutic agents has to be reduced, when a large volume is to be irradiated.

  Hepatopathy (veno-occlusive-disease, VOD) can be caused by actinomycin D alone. If VOD developed during pre-operative chemotherapy and post-operative irradiation of large parts of the liver should be avoided.

  In irradiation of the liver (15-20 Gy) liver function and thrombocytes have to be monitored (e.g. liver function tests), as an impairment may occur in the acute or chronic phase.

  Gastrointestinal side effects like diarrhea and vomiting may be observed during abdominal radiotherapy in particular if large volumes are treated. Symptomatic treatment for vomiting and for diarrhea is necessary including intravenous fluids are required. A diet free of lactose and saccharose and with low fat content is recommended for treatment of acute and late radiation enteritis.

  Impairmenty of bone and soft tissue growth mainly takes place years after radiotherapy and is most pronounced during growth spurts (113). The amount of impairment is dependent on radiation dose, irradiated volume, and age of the child and reveals as kyphoscoliosis. Hypoplasia (vertebral column, iliac bone, ribs, soft tissue of the flank), osteochrondroma.

  The impairment is expected to be smaller after low dose radiotherapy (15 Gy).

  Impairment of renal function induced by irradiation doses up to 12 Gy is not to be expected, as this radiation dose is far beyond the dose level at which renal dysfunction (e.g. as reduction in creatinin clearance) becomes probable. In combination with carboplatin and ifosfamide close follow-up renal failure is advised.

  Ovarian insufficiency is likely to occur after irradiation with doses about 15 Gy, if the true pelvis had to be included into the irradiated volume. Nevertheless, little is known about

ovarian tolerance doses for young girls. Hormonal function and fertility can probably be preserved if the ovarian dose can be kept below 2-3 Gy.

Impairment of spermatogenesis may occur even after scattered radiation doses above 50 to 100 cGy to the testes. Leydig cell function is much less radiosensitive and not influenced by such low scatter radiation dose.

Hypoplasia of the mamma is known to occur after doses about 1-3 Gy in the young child.

Reduction of lung volume and dynamic compliance can develop to some degree after radiotherapy to both lungs, more so in young children, because of insufficient growth of the rib-cage.

Cardiomyopathy in case of pulmonary irradiation, previous treatment with epirubicin or radiotherapy followed by this drug may increase the chance of this complication. Echocardiography with regular interval should be done to detect early toxicity (101).

II.6 Pathology Historical Background

All renal tumours diagnosed in children up to 18 years of age as well as typical renal tumours of childhood found in older adolescents should be registered. Typical renal tumours of childhood are nephroblastoma, clear cell sarcoma of kidney, rhabdoid tumour of kidney, and mesoblastic nephroma. Consultation for all cases will be provided without charge, and any use of material for teaching purposes or publication will credit the contributing pathologist (7, 14, 41, 63, 105, 119, 138, 142, 152, 161, 165, 172).

II.6.1 Role of the local pathologist in a participating centre

The pathologist has an essential role in both the clinical trial and the prospective study:

1. The local pathologist confirms the diagnosis of the renal tumour.
2. He/she classifies the tumour as low risk, intermediate risk or high risk.
3. He/she makes a precise evaluation of the abdominal stage of the tumour (even in children with stage IV disease, local staging is critical to determine the utilisation of radiotherapy). The pathologist should have information regarding pre- or intra-operative tumour rupture (from the surgeon) and clinical information regarding distant metastases. For the purpose of the Trial, please use the SIOP staging system.

Patients will be treated according to different therapeutic protocols *depending on tumour histology and stage.* As outlined elsewehere in the Trial protocol, low risk tumours, stage I, will be treated with no post-operative chemotherapy while high risk tumours will be treated more aggressively after surgery. Therefore, it is of the utmost importance for these tumours to be classified correctly – *in order to confirm the diagnosis prior to post-operative treatment, all low and high risk tumours should be sent for rapid review immediately after the operation*. Please submit a full set of H&E slides and one paraffin block from a viable tumour, accompanied by the SIOP Institutional Pathology Form and a copy of your report, to the Referring Pathologists.

II.6.2 Definitions of Nephroblastoma and its Sub-types, and Other Typical renal Tumours of Childhood

Based on the correlation between the histological features and survival, three prognostic groups of typical renal tumours of childhood were discerned in the previous SIOP Trials and Studies: low risk, intermediate risk and high risk tumours.

Mesoblastic nephroma, clear cell sarcoma of the kidney and rhabdoid tumour of the kidney represent separate entities from nephroblastoma but are typical renal tumours of childhood and are included in the SIOP classification and trial/study. Other, less common renal tumours

which may occur at any age including children should be also registered through the SIOP as they may provide a useful clue in our understanding of renal tumours.

The SIOP (Stockholm) Working Classification of Renal Tumours of Childhood has been recently revised to incorporate the results of the latest SIOP Trials and Studies and it will be followed in this Trial and Study (161). Some entities that existed in the previous classification, such as nephroblastoma with fibroadenomatous structures and highly differentiated epithelial nephroblastoma, have been either excluded or grouped with other subtypes. On the other hand, unlike in the previous classification where subtyping of nephroblastomas in the Intermediate risk group was not done but they were all labelled as non-anaplastic nephroblastomas, in this classification different types have been defined and will be studied prospectively although there will be no difference in their treatment in this Trial.

II.6.3 The Revised S.I.O.P. Working Classification of Renal Tumours of Childhood

II.6.3.1 Pre-treated cases

I. Low-risk tumours
- Mesoblastic nephroma
- Cystic partially differentiated nephroblastoma
- Completely necrotic nephroblastoma

II. Intermediate-risk tumours
- Nephroblastoma - epithelial type
- Nephroblastoma - stromal type
- Nephroblastoma - mixed type
- Nephroblastoma - regressive type
- Nephroblastoma - focal anaplasia

III. High-risk tumours
- Nephroblastoma - blastemal type
- Nephroblastoma - diffuse anaplasia
- Clear cell sarcoma of the kidney
- Rhabdoid tumour of the kidney

II.6.3.2 Primary Nephrectomy cases

I. Low-risk tunours
- Mesoblastic nephroma
- Cystic partially differentiated nephroblastoma

II. Intermediate-risk tumours
- Non-anaplastic nephroblastoma and its variants
- Nephroblastoma - focal anaplasia

III. High-risk tumours
- Nephroblastoma – diffuse anaplasia
- Clear cell sarcoma of the kidney
- Rhabdoid tumour of the kidney

Please note that nephroblastomas are treated according to their histological type and stage (and only stage I low risk tumours receive no postoperative therapy).

It is important to emphasise that for treatment purposes, in addition to anaplasia, only three major types of nephroblastoma need to be recognised: completely necrotic nephroblastoma (low risk tumours), blastemal (high risk tumour) and others (intermediate risk tumours), but pathologists are encouraged to record and enter in their reports a percentage of different components (regressive changes, blastemal, epithelial and stromal) as we will be prospectively analysing these features in order to identify those that might have further

prognostic significance. (Cystic partially differentiated nephroblastoma should be diagnosed on imaging studies and treated with surgery only).

Here follows a short description of the types of tumours that should be entered into these therapeutic trials and in the study. More detailed and extensive descriptions are given in the references given for each tumour.

II.6.3.3 Low Risk Tumours

- **Mesoblastic Nephroma.** Mesoblastic nephroma is a renal tumour that usually occurs in the first year of life. The oldest child with confirmed mesoblastic nephroma in the National Wilms' Tumor Study (NWTS) files was diagnosed at age of 29 months. Cases of 'mesoblastic nephromas' in older children have been shown to be Metanephric Stromal Tumours – a new entity recently defined by Beckwith. However, for both entities treatment is surgery and prognosis is excellent, so the distinction between them has no important therapeutic implications. (2, 11, 19, 47, 128, 157, 160).

  There are two histological subtypes of mesoblastic nephroma: the classical and the cellular type. The distinction between the two types has no implication for therapy so far. Classical mesoblastic nephroma is a monomorphous tumour composed of spindle cells with large, vesicular nuclei, noticable nucleoli and abundant cytoplasm. The cells are arranged in interlacing bundles and mitotic figures are usually present. The tumour-kidney border is irregular and long radial extensions (finger-like extensions) of tumour tissue into the adjacent renal tissue are a characteristic finding. Also, within the tumour small rests of connective tissue with entrapped tubules are usually seen. Cellular mesoblastic nephroma has a sharper, pushing tumour-kidney border, increased cellularity and numerous mitoses. Both types show infiltrative growth and may infiltrate the adjacent perirenal fat and spread into the renal sinus. Complete, wide surgical resection is the only recommened treatment for localised disease. Local recurrences and metastases have been described in a few cases, especially in children older than six months of age, although some children were < 1 month old at diagnosis. The vast majority of relapses occur within 12 months of nephrectomy and in about 70% of relapsed cases the tumour is of the cellular type.

  In the differential diagnosis, metanephric stromal tumour, blastemal and stromal nephroblastoma, clear cell sarcoma and rhabdoid tumour of kidney must be considered (in difficult cases, please consult excellent tables in 3rd series of AFIP Fascicle on 'Tumors of the kidney, bladder, and related urinary structures', 1994).

  Recently, cytogenetic abnormalities of chromosome 11 and a translocation involving chromosome 15 have been reported in cellular mesoblastic nephroma. The finding of ETV6-NTRK3 gene fusions and trisomy 11 has established a histogenetic link between cellular mesoblastic nephroma and congenital fibrosarcoma.
- **Cystic Partially Differentiated Nephroblastoma (CPDN).** CPDN is a distinct variant of nephroblastoma that usually occurs in children less than 2 years of age. *The histological criteria for making a diagnosis of CPDN* are as follows:
  - it is composed entirely of cysts and their thin septa;
  - the thin septa are the only 'solid' portion of the tumour;
  - the tumour forms a discrete mass, well demarcated from the non-cystic renal parenchyma;
  - the cysts are lined by flattened, cuboidal or hobnail epithelium; and
  - the septa contain blastemal cells in any amount, with or without other embryonal stromal or epithelial cell types.

Thus, variable differentiated glomeruli, tubules, mesenchyme, striated muscle, cartilage, fibrous tissue, and fat may be admixed with blastemal cells in septa. The presence of well-differentiated tubules only is not enough to make a diagnosis of this tumour and separate it from cystic nephroma. However, from a therapeutic and prognostic point of view there is no need to distinguish between CPDN and cystic nephroma as they are both treated with surgery only and both share the same, excellent prognosis. However, intermediate risk nephroblastomas may present with numerous cysts but they aslo contain solid areas and septa are usually thicker and show chemotherapy-induced changes. Beware that other renal tumours such as clear cell sarcoma and rhabdoid tumour may have a predominantly cystic appearance. (42, 44, 86)

- **Completely Necrotic Nephroblastoma**. Pre-operative chemotherapy given in SIOP trial patients results in so-called 'chemotherapy-induced change' in many nephroblastomas. Depending on their initial histological pattern, some nephroblastomas are completely or almost completely necrotic, while others show less marked or minimal/moderate changes. The relationship between the percentage of these chemotherapy-induced changes and prognosis has been shown in other tumours such as osteosarcoma as well as in a recent SIOP study on nephroblastoma in which completely necrotic nephroblastomas had excellent prognosis with 100% survival in all stages.

  The histological criteria for making a diagnosis of completely necrotic nephroblastoma are:

  - the absence of any viable tumour tissue on gross and microscopical examination of multiple blocks taken from different areas of a tumour, according to the recommended protocol; the presence of scattered mature tubules without is allowed as they may represent remnants of nephrogenic rests.
  - the presence of regressive and/or necrotic changes caused by chemotherapy.

  Although complete tumour necrosis makes histological subtyping of nephroblastoma impossible, 'ghost' tumour structures (mainly blastema, occasionally epithelial elements) can be recognised, and are helpful in distinguishing nephroblastoma from other renal tumours. In addition, the presence of nephrogenic rests, which are virtually never associated with non-Wilms' tumour and are generally not affected with chemotherapy, is a very reliable clue that the tumour has been a nephroblastoma before chemotherapy. Finally, it is well known that regression of other renal tumors such as clear cell sarcoma, rhabdoid tumor or renal cell carcinoma, is minimal to moderate under the actinomycin D - vincristine protocol, and their histological features can be easily recognised even in treated cases.

  The typical histological appearance of treated nephroblastoma is a mixture of necrosis, fibromyxomatous stroma containing lipid- and/or haemosiderin-laden macrophages, and haemorrhage. In some cases scattered mature tubules may be seen within necrotic areas – this may represent remnants of pre-existing rests and should not be regarded as viable tumour tissue. The main pattern of the necrotic area is coagulative-type necrosis of small round cells or tubules, with the majority of 'ghost' structures consisting of large sheets of small, pink, necrotic nuclei, consistent with coagulative necrosis of blastemal cells or tubules. (*If in doubt whether the necrotic tumour is a nephroblastoma, the reticulin staining may help to identify scarce epithelial or mesenchymal 'ghost' structures.*) The presence of identical changes in a lymph node is regarded as a proof of its involvement with a tumour and, therefore, it is very important to sample and microscopically examine all lymph nodes removed. Beware of Tamm Horsfall protein which is sometimes accompanied by discrete epithelium in a lymph node – this must not be interpreted as a metastasis (for other lesions and changes which may mimic lymph node metastases, see a paper by Weeks et al. (163)).

II.6.3.4 Intermediate Risk Tumours

Beckwith and Palmer's criteria for histological subtyping of nephroblastomas state that one component has to comprise at least 2/3 (66%) of a tumour mass for the tumour to be subclassified accordingly. However, pre-operative chemotherapy alters the original histological features of nephroblastomas and often results in areas of necrosis and regression. Therefore the criteria applicable to subclassification of primarily operated tumours have to be modified to take these changes into account. The reason that only viable tumour is taken into account when subclassifying nephroblastomas which are not completely necrotic is based on our previous studies which have shown that chemotherapy-induced changes are a prognostically favourable effect of treatment. On the other hand, the presence of blastema after pre-operative chemotherapy clearly indicates its non-responsiveness to chemotherapy and has been shown to be associated with poorer outcome. For all these reasons, we believe it is justified to modify the criteria for certain subtypes of nephroblastoma. We are aware that the assessment of percentage of necrosis/regression is subjective, but since it is very important for subclassification of nephroblastomas, it should be done on both gross and histological examination. (5, 17, 70, 165, 174)

Histological types of nephroblastoma from this group are described below, but a simple approach can be the following:

- Assess the percentage of necrosis/regressive changes
- If they comprise more than 2/3 of a tumour mass – it is a regressive type
- If they comprise less than 2/3 of a tumour mass – look for a predominant histological component and subclassify a tumour accordingly (blastemal, epithelial or stromal predominant). If no component is predominant, it is a mixed type.
- Even if you find focal anaplasia, try to subclassify the tumour as below.

In the group of intermediate risk tumours, five subtypes of nephroblastoma have been recognised as follows:

- **Nephroblatoma – Epithelial Type**

  The histological criteria for making a diagnosis of epithelial type nephroblastoma are as follows:
  - only the viable part of a tumour is assessed and it has to comprise more than 2/3 of a tumour mass;
  - the viable tumour consists of at least 2/3 of epithelial structures
  - the stromal component may comprise the rest of the viable tumour; and
  - scattered small foci of blastema comprising less than 10% of the tumour may occur (*the finding of a single, large nodule of blastema comprising about 10% of the viable tumour mass is not acceptable and such tumours should be subclassified as mixed subtype*).

  The epithelial elements are regarded as follows: (21, 24, 39, 85, 91, 165)

  a) *tubules* – spaces lined by columnar epithelial cells arranged in a fairly regular manner radially around the central space; cell margins are sharp, they have basal, crowded nuclei, and mitotic activity may be marked; tubules are usually back-to-back, with virtually no supporting stroma;

  b) *rosettes* – circular arranged tumour cells with elongated ovoid nuclei, but no central lumen is present;

  c) *papillary structures* – finger-like projections of a stroma covered with epithelial cells;

d) *glomerular structures* – tuft-like masses of malignant cells surrounded by a well-formed capsule or rather flattened tumour cells.

The stromal elements are regarded as follows: undifferentiated stromal cells, myxoid, fibroblastic, smooth muscle, skeletal muscle, adipose cells, cartilage and osteoid formations.

The presence of genuine anaplasia classifies the tumour as anaplastic nephroblastoma even if otherwise completely epithelial.

Epithelial nephroblastoma usually occurs in younger children (median age 9 months in a SIOP series), and about 80% of cases are in stage I. Beware of epithelial nephroblastoma in older children and search carefully for anaplasia.

- **Nephroblastoma – Stromal Type.** Stromal nephroblastoma represents subtype in which the stromal elements are a predominant component of the tumour. The fetal rhabdomyomatous nephroblastoma, which in the past was regarded as a nephroblastoma with better prognosis, is also included here. (98, 165, 167).

  The histological criteria for making a diagnosis of stromal type nephroblastoma are as follows:

  - *only the viable part of a tumou*r is assessed and it has to comprise more than 2/3 of a tumour mass;
  - the viable tumour consists of at least 2/3 of stromal elements;
  - the epithelial component may comprise the rest of the viable tumour; and
  - scattered small foci of blastema comprising less than 10% of the tumour may occur (*the finding of a single, large nodule of blastema comprising about 10% of the viable tumour mass is not acceptable and such tumours should be subclassified as mixed subtype*).

  The stromal elements are regarded as follows: undifferentiated, myxoid, fibroblastic, smooth muscle, skeletal muscle, adipose cells, cartilage, bone, and osteoid. Stromal differentiation may be induced by preoperative chemotherapy as a stromal type nephroblastoma is far more common in children who have recieved preoperative chemotherapy. It is likely that other tumour components, especially blastema, are destroyed by preoperative chemotherapy while stromal elements are chemotherapy resistant and may even further differentiate resulting in prominent skeletal muscle component, for example.

  Stromal nephroblastoma usually occurs in younger children and usually shows minimal to moderate chemotherapy induced changes since stromal tissue seems to be resistant to chemotherapy. Fetal rhabdomyomatous nephroblastoma is bilateral in 30% of cases.

- **Nephroblastoma – Mixed Type.** Mixed type nephroblastoma represents subtype in which none of viable component is predominant.

  The histological criteria for making a diagnosis of mixed type nephroblastoma are as follows:

  - only the viable part of a tumour is assessed and it has to comprise more than 2/3 of a tumour mass;
  - the viable tumour consists of blastemal and/or epithelial and/or stromal elements but none of them comprise more than 2/3 of the viable tumour.

  As for the other subtypes, please try to assess the percentage of different (viable) tumour component as well as the percentage of necrosis/regression.

- **Nephroblastoma – Regressive Type.** Nephroblastoma – regressive type is regarded as a tumour in which chemotherapy-induced changes comprise more than 2/3 of the tumour mass. Please note that assessment of percentage of necrosis/regression is done on both gross and histological examination, so blocks should be taken not only from viable parts of the tumour mass but also from those that show necrotic/regressive changes.

  The histological criteria for making a diagnosis of regressive type nephroblastoma are:
  - the presence of more than 2/3 of non-viable tumour tissue (regressive and/or necrotic changes caused by chemotherapy) on gross and microscopical examination of multiple blocks taken from different areas of a tumour, according to the recommended protocol
  - the viable tumour elements are histological components of nephroblastoma including blastemal, epithelial and stromal elements.

  The typical histological appearance of treated nephroblastoma is a mixture of necrosis, fibro-myxo-sclerotic stroma containing lipid- and/or haemosiderin-laden macrophages, and haemorrhage. The main pattern of the necrotic area is coagulative-type necrosis of small round cells, with the majority of 'ghost' structures consisting of large sheets of small, pink, necrotic nuclei, consistent with coagulative necrosis of blastemal cells.

- **Nephroblastoma with Focal Anaplasia - Nephroblastoma with Focal Anaplasia *focal anaplasia*** has been moved into the Intermediate risk group since both NWTS and SIOP studies have shown that it has the same prognosis as non-anaplastic nephroblastomas (other than blastemal type, in the SIOP trials). Diagnostic criteria for focal anaplasia have been described with diffuse anaplasia (see below).

II.6.3.5 High risk Tumours

- **Nephroblastoma – Blastemal Type.** This nephroblastoma type has been moved into the high risk tumours but only if diagnosed after pre-operative chemotherapy. The reason for this change is based on the results of previous SIOP trials showed that tumours with chemotherapy resistant blastema had a very bad prognosis and would require more aggressive treatment. In cases diagnosed after primary nephrectomy, blastemal nephroblastoma remains in the Intermediate risk tumours.

  The histological criteria for making a diagnosis of blastemal type nephroblastoma are as follows:
  - only the viable part of a tumour is assessed and it has to comprise more than 2/3 of the tumour mass;
  - at least 2/3 of the viable tumour consists of blastema
  - other components of nephroblastoma may be present in varying proportions.

  The blastemal elements are regarded undifferentiated round or elongated cells which are usually closely packed and show no evidence of epithelial and/or stromal differentiation. There are several distinctive patterns in which blastemal cells may occur and it is not uncommon to find more than one pattern in the same tumour. They include the diffuse, serpentine, nodular, and basaloid patterns but they are of no prognostic or therapeutic significance (for detailed criteria for different blastemal patterns, please see 3rd series of AFIP Fascicle on 'Tumors of the kidney, bladder, and related urinary structures', 1994).

- **Nephroblastoma with Anaplasia.** Anaplasia was recognised as an unfavourable histological feature of nephroblastoma in earlier trials. The histological criteria for making a diagnosis of anaplastic nephroblastoma are the presence of all three criteria for anaplasia including:

- the presence of atypical tri/multipolar mitotic figures;
- marked nuclear enlargement, with diameters at least three times those of adjacent cells
- the presence of hyperchromatic tumour cell nuclei.

Anaplasia may occur in the blastemal, epithelial or stromal component of nephroblastoma and it can be focal or diffuse. The recent (topographic) definition of focal anaplasia emphasizes the distribution of anaplasia which has to be sharply demarcated within the primary tumour. This proved to be of prognostic significance in both primarily operated and prenephrectomy treated cases. (18, 46, 62, 159, 174)

- **Focal anaplasia** has now been defined as the presence of a clearly defined focus of a few discrete, sharply demarcated small foci within a primary intrarenal tumour, without evidence of anaplasia or prominent nuclear atypia in extrarenal tumour sites.
- **Diffuse anaplasia** is defined if any of the following are present:
  - non-localised anaplasia, and/or anaplasia beyond the original tumour capsule;
  - anaplastic cells present in intrarenal or extrarenal vessels, renal sinus, extracapsular invasive sites, or metastatic deposits;
  - anaplasia is focal, but nuclear atypia approaching the criteria for anaplasia (so-called 'unrest nuclear change') is present elsewhere in the tumour;
  - anaplasia that is not clearly demarcated from non-anaplastic tumour; and
  - anaplasia is present in a biopsy or other incomplete tumour sample.

This topographic definition of focal anaplasia makes it mandatory that pathologists carefully document the exact site from which every section is obtained (e.g. on a diagram, specimen photocopy, and/or photograph of the gross specimen). Please use a pre-prepared diagram in the SIOP Institutional Pathology Form F4 or a photograph.

Anaplasia occurs in about 5% of patients with nephroblastoma. Preoperative chemotherapy does not obliterate or produce anaplasia but it makes its recognition easier since non-anaplastic areas are destroyed by chemotherapy while anaplastic foci remain unchanged. This provides further support to the hypothesis that anaplasia represents more resistant rather than a more aggressive cell line. The age distribution of anaplastic nephroblastoma differs from non-anaplastic nephroblastoma: anaplasia never occurs in the first six months of life, it is very rare between 6-12 months (1-2%), median age at diagnosis is 61 months and >50% of children are over five years of age (for non-anaplastic nephroblastoma median age is 45 months, and 25% of children are over five years of age).

Although the criteria for anaplasia have been well established, it still represents a diagnostic problem resulting in either missed or 'overdiagnosed' cases, while only in rare instances it is confused with other renal tumours. It is important to bear in mind that all three criteria for the diagnosis of anaplasia have to be met and that some histological changes may mimic anaplasia including calcification, fused or smudged masses of nuclear chromatin due to technical artefact, stain precipitate, circulating megakaryocytes, overlapping cells in thick sections, and bizarre nuclei resulting from chemotherapy with the formation of hyperchromatic multinucleated and bizarre

macronucleated skeletal muscle cells in response to injury. However, the diagnosis of anaplasia in the skeletal muscle must be made if atypical mitoses and other histological criteria are present.

- **Clear Cell Sarcoma of the Kidney.** This distinctive tumour comprises 5% of primary renal tumours of childhood. It is extremely rare in the first six months of life and in young adults, and the majority of patients are between 2 and 3 years of age. There is a male predominance, but no association with chromosomal defects, genetic abnormalities or specific malformations and syndromes has been reported. Unlike nephroblastoma, CCSK is always unilateral and unicentric.

  Histologically, this tumour has a deceptively bland appearance and many histological subtypes. The classical pattern has a uniform appearance of a diffuse growth of relatively small cells with normochromatic nuclei, inconspicuous nucleoli, pale staining cytoplasm, and ill-defined cell membrane. In only 20% of the cases do the tumour cells have clear cytoplasm. The most characteristic feature is a peculiar vascular pattern consisting of arborising blood vessels that create an alveolar or trabecular pattern (best seen with the reticulin stain). (1, 8, 110, 128, 161)

  The classical pattern of CCSK is relatively simple to diagnose, but others including the myxoid, sclerosing, cellular, epithelioid, palisading, spindle cell, storiform, and anaplastic pattern can cause problems in reaching the diagnosis. In some CCSKs, there can be extensive hyalinisation and these tumours may be confused with cases of nephroblastoma with sclerosis due to pre-operative treatment, or rhabdoid tumour. In differential diagnosis blastemal nephroblastoma, mesoblastic nephroma, PNET and rhabdoid tumour must be considered (in difficult cases, please consult excellent tables in 3rd series of AFIP Fascicle on 'Tumors of the kidney, bladder, and related urinary structures', 1994, and the paper by Argani et. al., (1)).

  The histogenesis of the tumour is uncertain. The tumour cells are only positive for vimentin and are generally negative for cytokeratin, factor VIII associated antigen, epithelial membrane antigen, desmin, and S100 protein.

- **Rhaboid Tumour of the Kidney.** Rhabdoid tumour of kidney (RTK) is rare, constituting 2% of paediatric renal tumours. It typically occurs in early childhood, with about 80% of patients younger than 2 years, while it is extremely rare after 5 years of age. Two characteristic associations of RTK are hypercalcaemia and the development of synchronous or metachronous primary brain tumours. On the other hand, it is never associated with conditions predisposing to nephroblastoma or with nephrogenic rests. (15, 158, 162, 164)**. *Histological criteria for diagnosis of rhabdoid tumour*** include the finding of its characteristic histological features and unique immunohistochemical profile. Typical histological features comprise non-cohesive sheets of cells with abundant eosinophilic cytoplasm and *large eccentric nuclei with prominent eosinophilic central nucleoli* - these are regarded as the most characteristic feature of the tumour and they are always present at least in some areas of the tumour. Another characteristic feature is the presence of *large oval intracytoplasmic hyaline inclusions* composed of whorled masses of intermediate filaments. Both of these features may only be focal, and should be specifically looked for in any undifferentiated renal tumour of childhood. In addition to the classical pattern of rhabdoid tumour, many other patterns have been described including sclerosing, clear cell sarcoma-like, epithelioid, spindled, lymphomatoid, vascular, pseudopapillary and cystic patterns. Immunohistochemistry shows consistent positivity of tumour cells for vimentin with frequent co-expression of cytokeratin, while many other markers including epithelial membrane antigen, S-100 protein, neurofilaments, neuron-specific enolase, desmin, myoglobin, alpha-1-antichymotrypsin have been reported but are not found consistently. CD99 (Mic-2) positive staining may be seen too. In some cases abnormalities of chromosome 22 and 11p13 have been described.

- **Nephrogenic Rests.** Nephrogenic rests are foci of embryonal cells which persist after 36 weeks of gestation and they are considered as potential precursors of nephroblastoma. They have been found not only in 25-40% of patients with nephroblastoma but also in 1% of routinely examined perinatal postmortem kidneys. However, they have not been described associated with other typical renal tumours of childhood and their finding in problematic cases should be regarded as a very useful clue that the tumour is nephroblastoma. Two main types of nephrogenic rests have been recognised: perilobar and intralobar rests. They can be further subclassified as dormant, sclerosing, or hyperplastic, and all these appearances may be present in an individual case. The rests may regress to fibrous tissue or progress to nephroblastoma. Hyperplastic rests may be difficult to distiguish from a small nephroblastoma but it is usually of no therapeutic significance since both hyperplastic rests and nephroblastoma should be treated (please, see elsewhere in the Protocol). Perilobar rests occur in hemihypertrophy and Beckwith-Wiedemann syndrome while intralobar rests are associated with WAGR and Denys-Drash syndromes. (10, 12).
- **Other tumours included in the study:** In addition to more common renal tumours of childhood discussed above, there are numerous other tumours which may occur at any age. Although these tumours are not entered in the Trial, they should be registered and submitted as they may provide important information in our understanding of renal tumours in general. These include:
  - Metanephric tumours (metanephric stromal tumour, metanephric adenofibroma, metanephric adenoma)
  - Adenomas (all other types)
  - Cystic nephroma
  - Renal cell carcinoma (all variants)
  - Transitional cell carcinoma
  - Neuroepithelial tumours (renal neuroblastoma, renal PNET, renal carcinoid)
  - Miscellaneous sarcomas (without evidence of blastemic cells and/or epithelial component in five different blocks)
  - Renal lymphoma
  - Angiomyolipoma
  - Other tumours (adrenal tumours, teratoma) and lesions (xanthogranulomatous pyelonephritis, etc), if preoperative chemotherapy for nephroblastoma has been given
  - Metastases from other sites

II.6.4 Differential Diagnosis of Renal Tunours of Childhood

The results of the SIOP 9 and SIOP 93-01 trials showed that there was a number of cases of both low and high risk tumours that were misdiagnosed including cystic partially differentiated nephroblastoma, highly differentiated epithelial type nephroblastoma, anaplastic nephroblastoma, clear cell sarcoma and rhabdoid tumour of the kidney. Since many of them were seen by the Panel retrospectively, this resulted in either over-treatment (for low risk tumours) or under-treatment (for high riks tumours). As groups of low and high risk tumours have changed in this Trial, it has become even more important to reach a correct diagnosis before any post-operative treatment is administered.

There are some clinical, macroscopical and histological features of renal tumours of childhood which might be a useful clue in reaching a correct diagnosis.

Age at diagnosis is a rather reliable criterion. Anaplastic nephroblastoma has never been described in the first six months and is extremely rare in the first year of life, but after 5 years of age it comprises 10% of nephroblastomas. Clear cell sarcoma of kidney hardly occurs in

the first 6 months of life, while mesoblastic nephroma and rhabdoid tumour of kidney are extremely rare in children over 3 years of age.

Grossly, many renal tumours may show areas with *cysts* but only CPDN and cystic nephroma are entirely cystic neoplasms, with no solid areas. Nephroblastoma is the only typical renal tumour of childhood which may be *bilateral* (in 5% of cases) or *multifocal;* There are some unique features of nephroblastoma which are very useful in distinguishing it from other renal tumours:

- *nephrogenic rests* are commonly present in nephroblastoma but not in other tumours (there is only one report of nephrogenic rests associated with mesoblastic nephroma and CCSK, respectively)
- the presence of *skeletal muscle, adipose tissue* and genuine *neoplastic tubules* has only been seen in nephroblastoma (although fat may be present in metanephric stromal tumours, other features should be sufficient to make a correct diagnosis).
- nephroblastoma has been diagnosed in a child with a *syndrome predisposing to nephroblastoma* (WAGR, Beckwith-Wiedemann, Denys-Drash syndrome) while mesoblastic nephroma is the only other renal tumour that has occasionally been described with Beckwith-Wiedemann syndrome.

When in doubt about either the histological type or the stage, please send a full set of histological sections to the referring pathologist immediately.

II.7 Study of the Nephrectomy Specimens

The *intact* surgical specimen should be presented to the pathologist *without being opened by the surgeon,* and should be accompanied with a report of the operation (form F3) with sufficient information necessary for correct staging.

II.7.1 Handling the fresh specimen, step by step

- *Weight, measure* and *photograph* the whole specimen. Look carefully for ruptures and fissures and locate any suspicious areas and/or ink it in different colours from the rest of the specimen. Decapsulation makes determination of growth beyond the capsule impossible and therefore should not be done.
- Look for and dissect the peri-renal and perihilar *lymph nodes*. Block these separately recording the site. (These are rare).
- *Identify renal vein, artery* and *ureter* and take transverse section block of each near the resection margin.
- *Ink* the surface of the whole specimen (or at least areas in which radicallity is dubious) and renal sinus with Indian ink and let it dry *before* opening the specimen. This is a critical step and should always be done as otherwise it might be impossible to stage the tumour correctly and give adequate therapy.
- *Open* by a longitudinal incision to bivalve the specimen and reveal the tumour and its relation to the kidney, capsule, and renal sinus.
- *Photograph* the cut surface, record macroscopic appearance. *Measure* the size of the tumour. It is crucial to *assess the percentage of a necrotic tumour* (this percentage has to be filled in on the Form F4) and also to describe and photograph the multicystic cut surface, if present.

- Take *fresh material* (tumour and kidney) for special studies (snap freezing in liquid nitrogen, cytogenetics, flow cytometry - see molecular biology chapter 14).
- The specimen should be *fixed* in 4% buffered formalin for 24 to 48 hours according to the usual procedure of the laboratory. Several additional cuts can be made parallel to the initial cut to divide the specimen into "slabs" for better fixation. (*Alternatively, instead of parallel longitudinal sections, you may find that making horizontal sections and sampling the tumour in this way will give you a better view of the renal sinus and a tumour-sinus relationship*.)

- **The samples for histological examination should include**:
  - the macroscopically different areas of the tumour (it is advised to take at least one block per cm of the largest diameter of the tumour, do not forget to take blocks from grossly necrotic areas, too); mostly from the periphery rather than from the central areas of the tumour;
  - dubious areas have to be marked by the surgeon and need special attention of the pathologist (they have to be marked with Indian ink or methylen blue);
  - sinus lymph nodes when present;
  - other lymph nodes.
  - renal pelvis and pelvic fat, ureter and sinus vessels; especially the renal vein should be inspected for evidence of tumour thrombus; if present, it is critical to assess whether it is completely resected;
  - each nodule away from the main mass (in multifocal tumours);
  - tumour-kidney interface
  - tumour-kidney capsule
  - areas of the capsule that are suspected to be invaded by the tumour;
  - areas of perirenal fat suspected for tumour infiltration (important for assessment whether the tumour is completely resected);
  - areas of adhesions of the tumour to surrounding tissues;
  - at least 2 blocks of the normal kidney and blocks from abnormal looking areas in the remaining renal tissue.

All the samples should be numbered and their sites recorded as well as all other samples taken at the time of operation, i.e. adrenals, lymph nodes and various biopsies.

→ Please use a pre-prepared diagram in the SIOP Institutional Pathology Form F4 or a photograph.

II.7.2 Staging

Stage is one of the most important therapeutic and prognostic criteria for renal tumours. It has been shown in all multicentre trials that staging still represents a major problem, partly because of the fact that renal tumours are usually very large at nephrectomy and often it is very difficult to assess their relationship with normal renal anatomical structures such as the renal capsule and the renal sinus. It is absolutely critical to take blocks from all sites that are important for staging and to carefully document the site from which each block is coming.

→ Please use a pre-prepared diagram in the SIOP Institutional Pathology Form F4 or, preferably, a photograph and mark the sites from which blocks have been taken).

→ Please remember that local (abdominal) staging of primary tumour is done following prenephrectomy chemotherapy and it is very important even in stage IV cases. The presence/absence of metastases is evaluated at presentation, on the basis of imaging studies.

Here follow the criteria for staging:

- **Stage I**
  - The tumour limited to kidney or surrounded with a fibrous pseudocapsule if outside of the normal counters of the kidney, the renal capsule or pseudocapsule may be infiltrated with the tumour but it does not reach the outer surface, and it is completely resected (resection margins 'clear')
  - The tumour may be protruding ('bulging') into the pelvic system and 'dipping' into the ureter (but it is **not** infiltrating their walls)
  - The vessels of the renal sinus are not involved
  - Intrarenal vessel involvement may be present

  → **Fine needle aspiration or percutaneous core needle biopsy ('tru-cut') do not upstage the tumour but the size of gauge should be mentioned to a pathologist.**

- **Stage II**
  - The tumour extends beyond kidney or penetrates through the renal capsule and/or fibrous pseudocapsule into peri-renal fat but is completely resected (resection margins 'clear')
  - Tumour infiltrates the renal sinus and/or invades blood and lymphatic vessels outside the renal parenchyma but it is completely resected
  - Tumour infiltrates adjacent organs or vena cava but is completely resected
  - The tumour has been surgically biopsied (wedge biopsy) prior to preoperative chemotherapy or surgery

- **Stage III**
  - Incomplete excision of the tumour which extends beyond resection margins (gross or microscopical tumour remains post-operatively)
  - Any abdominal lymph nodes are involved
  - Tumour rupture before or intra-operatively (irrespective of other criteria for staging)
  - The tumour has penetrated through the peritoneal surface
  - Tumour implants are found on the peritoneal surface
  - The tumour thrombi present at resection margins of vessels or ureter, transsected or removed piecemeal by surgeon

- **Stage IV**
  - Haematogeneous metastases (lung, liver, bone, brain, etc.) or lymph node metastases outside the abdomino-pelvic region.

- **Stage V**
  - Bilateral renal tumours at diagnosis. Each side should be substaged according to above classifications.

→ **If in any doubt about a tumour's stage, please send it for urgent review to the referring pathologist.**

National Reference laboratory

Ivo Leuschner
Tel:49 - 431 - 597 3450Institut für Paidopathologie
Fax:49 - 431 - 597 3486Klin. der Christian-Albrechts-Universitat
Email:Michaelisstrasse 11
24105 Kiel
FR German

II.8 Statistical Considerations

II.8.1 Statistical Considerations for SIOP 2001

As stated in section 2 of the protocol, the SIOP 2001 Nephroblastoma clinical trial and study continues to:

- Collect prospectively detailed information on patient and tumour characteristics in relation to therapy and prognosis;
- Explore the possibilities of further reducing adjuvant treatment in Wilms tumours in order to minimise acute and late toxicity without jeopardising recurrence and survival.
- Results and experiences from previous SIOP studies in nephroblastoma are used to plan therapy and design new studies for specific risk groups.

The following schedule of risk group related therapy would be applied:

Risk	Stage	Treatment (Post-operative)
Low risk	I	No further treatment
	II, III	AV-2
Intermediate risk	I [a]	Short arm SIOP 93-01 (AV-1)
	II, III [b]	Randomisation +/- DOX (III+RT)
High risk	I	Three drugs (AVD)
	II, III	High risk protocol + RT

[a] GPOH protocol will treat patients with intermediate risk (excluding epithelial and stromal predominant) and pre-operative volume >500 ml as stage II with DOX

[b] GPOH protocol will treat patients with intermediate risk (excluding epithelial and stromal predominant) and pre-operative volume >500 ml according to High Risk protocol

- **Intermediate risk, stage II/III.** The study question in the SIOP 2001 study, allowing randomisation, is the post-operative treatment regimen in intermediate risk stage II and III patients.

  The question is whether Doxorubicin can be excluded from the post-operative chemotherapy combination without deterioration of currently achievable event-free survival (EFS).

  Stage II and stage III patients receive both the same chemotherapy regimen. Stage III patients receive post-operative abdominal radiotherapy in addition.

- **Sample size.** The required sample size in this study will be based on results from the SIOP 93-01 study and will be calculated using the methodology of establishing equivalence between treatments[i]. The null hypothesis of non-equivalence is to be tested (rejected): H_o: elimination of Doxorubicin treatment reduces the 2-year event-free survival to an unacceptable proportion ($\Delta \geq 10\%$) versus the alternative hypothesis (H_a) of equivalence. (126)

  The probability α, of wrongly accepting the hypothesis that both treatment regimens are equally effective, will be limited to 5%. The probability of not recognising equivalence (wrongly accepting non-equivalence) will be accepted to a level of 20% (power 80%). The estimated 2-year overall event-free survival in the SIOP 93-01 study was 85% (2/3 stage II (2-year EFS 88%), 1/3 stage III (2 year EFS 78%)). Thus, present considerations result in 158 patients needed in each treatment group, which translates in an overall sample size of 320 patients in total. Based on number from the SIOP 93-01 study it is estimated that it will take about 7 years to accrue this number of patients and another 2 years of follow-up is required for analyses at full power. (111, 112)

- **Randomisation.** Patients can be randomised only within two weeks after surgery (and confirmation of the stage of disease). Randomisation is performed centrally by computer according to the minimisation technique. Patients will be stratified by institute and pathological stage. After receiving written informed consent, the local physician can call the group representative trial office (GPOH, SFOP, UKCCSG and SIOP Amsterdam). The trial office will contact the central computer either through the Internet or by calling to the Netherlands Cancer Institute.

- **Interim analyses.** During the period of intake to the study, interim analysis of event-free survival will be supplied, in strict confidence, to a Data Monitoring Committee, along with other analysis that the committee may request. In the light of these interim analyses, the Data Monitoring Committee will advise the principal investigators whether in their view the trial has provided enough evidence that for all, or some types of patients one particular treatment is indicated or contraindicated in terms of net difference in survival. A sequential analysis, applying O'Brien-Fleming boundaries with an overall significance level of 0.05 can be used to statistically guide the advice[ii]. The principal investigators can then decide whether to modify the intake to the study. Unless this happens, the principal investigators and the collaborators will remain ignorant of the interim results.

- **Main statistical analyses.** The primary efficacy variable is 'event-free survival' (EFS). All randomised patients will be included in the analyses and presented according to the treatment they were randomised to receive ('intention-to-treat' (ITT)). All patients who prematurely withdraw from the study for any reason will be actively followed up.

  The two proportions of failures (recurrences or deaths) will be compared with the normal approximation test of Newcombe-Wilson[iii].

  When, at the final analyses, including a 2-year follow-up of all patients, the observed confidence limit of the differences is less than 10%, the conclusion will be that the two treatment regimens are equivalent.

  Analyses of the 'per protocol population' (all patients from the ITT population adhering to the protocol and analysed according to the treatment that they received) will be performed for the primary efficacy endpoint only in order to assess the robustness of the results.

- **Stage I, low risk.** As with the SIOP 93-01 protocol, no stopping rules will be designed for patients with stage I, low risk disease. However, failures, when they occur, have to be reported immediately to the secretariat in Amsterdam. The 95% confidence interval EFS rate will be estimated at the time of each event and provided to the Data Monitoring Committee.

- **Stage I, intermediate risk.** Patients with stage I, intermediate risk will receive the short chemotherapy regimen (4 weeks) that was suggested in the SIOP 93-01 trial. An interim analysis at the end of the accrual period in 2000 did not show any in-equivalence between the 4 weeks schedule compared to the 8 weeks schedule in terms of event-free survival. However, at the time of the preparation of the SIOP 2001 protocol the full analyses (including at least 2 year follow-up of all patients) of the trial in this patient group were not yet available. Therefore, in the SIOP 2001 protocol these patients will be followed up carefully and in 2002, when full analyses of all SIOP 93-01 trial patients will become available, the newly collected data will be analysed and evaluated against the 95% confidence intervals of the event-free survival of the corresponding patients of the SIOP 93-01.

- **Other risk groups.** In general all other risk groups (combinations of stage and histology) are small. EFS and overall survival with corresponding confidence intervals will be estimated for all of these groups. The results will be reported and considered in the light of previous studies and new developments.

II.8.2 Statistical Considerations for the ACGT Nephroblastoma Trial

As stated above patients within the ACGT Nephroblastoma trial will be treated according to the SIOP 2001 protocol and followed. In addition these patients will be analysed for humoral immune response against nephroblastoma specific antigens according to the scenario described in section 15.

At four differnet timepoints serum of patients will be analyzed for an humoral immune response against nephroblastoma antigens:

timepoint 1:at diagnosis, without treatment

timepoint 2:after preoperative chemotherapy, before surgery

(not available in primarily operated patients)

timepoint 3:after surgery

timepoint 4:at the end of treatment

The whole scenario is described in chapter 15.

The following 6 questions will be addressed in this scenario and answered:

---

1. Is the antigen pattern at diagnosis pathognomonic for Wilms tumor?
2. Is the antigen pattern at diagnosis pathognomonic for each histological subtype of Wilms tumor?
3. Is there an expression of those genes in the tumour coding for the antigens found in step 2 of the scenario?
4. Is it possible to define a specific pattern of antigens that correlate with the response to preoperative chemotherapy?
5. Is it possible to define a pathognomonic pattern of antigens that correlates with the outcome of the patients?
6. Does the individual pattern of antigens reflect the course of disease over time ? or Can this antigen pattern or single antigens be used as a tumour marker ?

The purpose of the ACGT nephroblastoma trial is to analyze at least the serum of 200 patients for the humoral immune response against nephroblastoma antigens at the four different time points. By the recruiting rate of about 100 patients / year in Germany, this will last for 2 to 3 years.

II.8.3 SIOP 2001 Biological Studies

II.8.3.1 Prognostic factors in Wilms tumour

Two clinical factors are established as conferring adverse prognosis in Wilms tumour, namely, histological subtype (anaplasia) and advanced tumour stage. Although many molecular abnormalities have been proposed to be associated with adverse outcome in Wilms tumour, none is yet used to stratify treatment. These include allele loss at chromosomal regions within 16q, 1p and 22q, p53 mutation or overexpression, telomerase activity, gain of 1q, expression of TRKB and certain multidrug resistance genes etc. One of the aims of the NWTS 5 study was to analyse, in a prospective fashion, the prognostic significance of allele loss on 16q, 1p and DNA ploidy in favourable histology Wilms tumour. The results o fthis trial show that LOH of 1p and 16q are of prognostic value (68). In the ongoing COG trials in North America LOH 1p and 16q are used as stratification parameters for treatment.

Expression profiling of nephroblastoma, as well as analysis of metabolic pathways are under investigation in different laboratories all around the world, to establish new prognostic markers and to help to find new therapeutic regimens. Results are still under discussion.

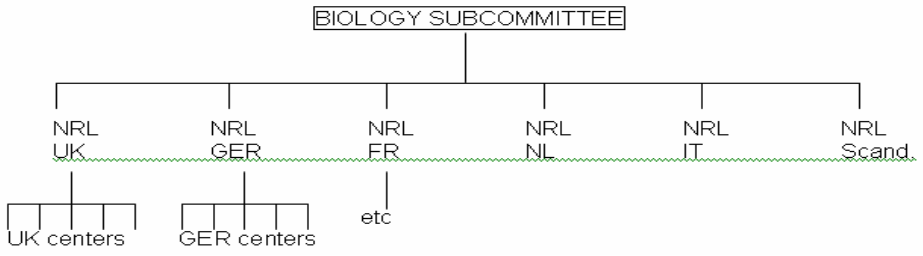
(168, 169, 170, 171). The SIOP 2001 nephroblastoma trial and study has the unique opportunity to assess prognostic factors that relate to response to pre-operative treatment. Two factors that are being analysed in the current study are histological appearance and tumour volume following pre-operative chemotherapy. It is important that the relation of these to molecular markers is studied so that the results of SIOP 2001 can be compared with those of NWTS 5.

II.8.3.2 Aims

- *Prospective testing of biological prognostic markers.* To correlate allele loss at 16q, 1p and other chromosomal regions of interest with relapse-free and overall survival of children with Wilms tumour treated within the SIOP 2001 nephroblastoma trial and study.
  - To correlate the above allele losses with clinical risk factors defined following pre-operative chemotherapy (i.e. histological appearance and tumour volume).
  - To establish, on a national basis, a Wilms tumour biological samples bank containing frozen tumour and normal kidney and/or blood, that will be available to conduct further research into molecular prognostic factors and Wilms tumour biology.
- *Additional research projects using the SIOP-WT study structure.* Further studies will be conducted on a national or international basis according to the research interests of participating institutions, subject to approval by the SIOP Wilms' tumour Biology Committee.
  - ⇨ **Familial Wilms tumour:** All cases of familial Wilms tumour should be notified to the SIOP data centre. Further studies, including pedigree evaluation and blood sampling will be at the discretion of the local clinician, who is encouraged to contact one of the following interested laboratories (Dr. Pritchard-Jones & Rahman, UK; Dr Jeanpierre, France; Dr Gessler, Germany).
  - ⇨ **Overgrowth syndromes, particularly Beckwith-Wiedemann syndrome:** High resolution molecular studies of genes on chromosome 11p15 are undertaken by Dr Marcel Mannens, Amsterdam, who is happy to receive diagnostic and research samples.
  - ⇨ **Denys-Drash syndrome and Wilms tumour with associated genitourinary malformation:** Drs. Jeanpierre and Fournet, Hopital Necker, France, are interested in analysing all new patients in this category for WT1 mutations. They maintain a WT1 mutational database at http://www.umd.necker.fr
  - ⇨ **Bilateral Wilms tumour and nephrogenic rests:** Drs. Jeanpierre and Fournet, Hopital Necker, France, are interested in receiving samples of tumour, nephrogenic rests and normal kidney, to look for the molecular bases of this association.
  - ⇨ **Humoral immune response against specifiv nephroblastoma antigens:** This will be studied by the group of Drs. Norbert Graf, Alexander Hoppe and Eckart Meese from the University of the Saarland within the ACGT Nephroblastoma trial.

II.8.3.3 Biology Subcommittee

There is a biology subcommittee. All responsible persons of the National Reference Laboratory (NRL) are member of this committee. Representatives of participating centers are welcome to the meetings of this committee if they show their interest. The committee is chaired by one of the NRL representatives. The subcommittee chooses their own chairperson. Research laboratories performing specific studies are welcome to discuss how cooperation and logistics can be organised by the committee.



II.8.3.4 Samples Required

- *Tumour*: Two pieces (0.5 - 1 cm^3 each) of morphologically different parts of the tumour should be sampled and snap frozen in liquid nitrogen or at –70°C. If a biopsy is performed prior to commencing pre-operative chemotherapy, then a sample of this should also be frozen, if adequate tissue is available.
- *Adjacent normal kidney*: two pieces (0.5 – 1 cm^3) snap frozen in liquid nitrogen or at –70°C.
- If present, *nephrogenic rests* should be sampled as above.
- 10 ml peripheral blood in EDTA (if national procedure for storage available).
- Samples should be stored at –70°C or under liquid nitrogen until transported to the appropriate national research laboratory on dry ice.
- *5 ml peripheral blood* for humoral immune response at 4 different timepoints (t1: at diagnosis, t2: after preoperative chemotherapy, t3: after surgery, t4: at the end of treatment).

II.8.3.5 Sample handling

The time interval between removal of the tumour and the freezing of the samples should be as short as possible and certainly not exceed a period of 30 minutes. Wilms tumours may contain extensive areas of necrosis following chemotherapy. Pathologists should ensure that samples for storage are taken from areas of grossly viable tumour. Adjacent samples should be studied for pathology/histology.

→ **It is of utmost importance, that before removing tissue for biological studies, the local tumour stage is stated by the local pathologist.**

II.8.3.5 Methodology

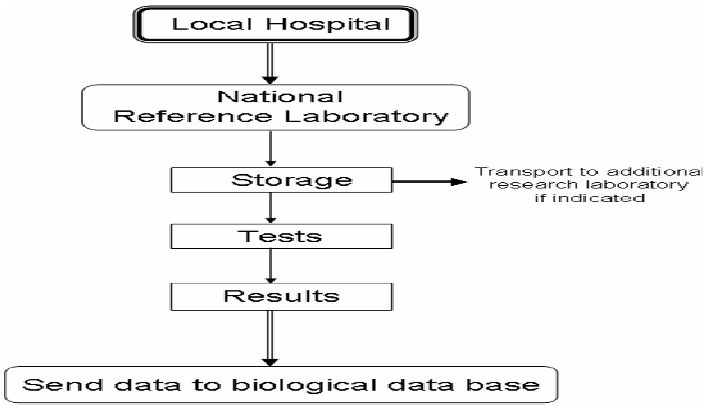
Paired normal and Wilms tumour DNA samples will be investigated for allele loss (LOH) using a panel of highly polymorphic microsatellite markers by polymerase chain reaction (PCR). LOH for an individual allele will be defined as >50% reduction in band intensity, as determined by densitometry. Tumour LOH for a specified chromosomal region will be defined by the loss of more than 1 adjacent marker. A set of common markers will be used by all participating laboratories, with blinded sharing of samples for quality control. The nature and number of markers to be used will be decided following preliminary studies across the chromosomal regions of interest and using data provided by the NWTSG.

⇨ **Patient outcome**: Molecular analysis will be 'blind' without knowledge of the status of the patient, other than diagnosis, by the researcher. Once the molecular analysis is complete, analysis of clinical correlations will be undertaken by the SIOP nephroblastoma data centre and, where appropriate and agreed by the Biology Committee, national subsets may be analysed by their own national data centres.

II.8.3.6 Specimen Routing

- **Operating theatre**
  - surgeon takes out the specimen
  - surgeon fills in the surgical form
  - surgeon sees to it that specimen will be taken to pathology laboratory
- **Pathological laboratory**
  - pathologist deals with the specimen with special care for staging!
  - she/he sees to it that viable tumour tissue is sampled according to the protocol for biological study
  - this tissue is snap frozen within two hours after resection and stored at -80°C
  - pathologist makes diagnosis and fills in pathological form
  - pathologist decides whether short term central review is necessary
- **Biology laboratory**
  - snap frozen tissue is taken to tissue bank
  - biologist arranges for registration
  - also blood samples from patient and parents are sent to this lab by the clinician and is stored at this site
  - material is sent to a National Reference Laboratory if relevant (see "Further routing").

- **Local Hospital …**



II.9 ACGT Nephroblastoma Scenario: Identification of Nephroblastoma antigens and determination of the seroreactivity

II.9.1 Scenario description

Immunogenic tumor-associated antigens have been reported for a variety of malignant tumors including brain tumors, prostate, lung and colon cancer. In a first step, immunogenic Wilms tumor associated antigens will be identified by immunoscreening of a cDNA expression library spotted on a Polyvinylidenfluorid (PVDF) membrane. Five sera in total from Wilms tumor patients of all three risk groups will be combined and diluted to a final concentration of 1:1000. Antigen-antibody complexes are detected with horseraddish-conjugated anti-human IgG antibody, followed by chemiluminescent detection with ECF™. This first step will identify those antigens that show reactivity against serum antibodies of patients with Wilms tumor and not with healthy individuals. Only those antigens, that react with this pooled serum and not healthy serum (newly identified Wilms tumor antigens), will be used in the following experiments.

Serum from a specific patient will be tested against these newly identified Wilms tumor antigens. As a result in each patient there will be a specific pattern of antigens found, found by the reaction between tumour associated antigen and serum antibody measured by chemiluminescent detection. This specific pattern (different antigens) will be used as a result of the experiment. This pattern will be correlated to the histological subtype of the tumor, the gene expression profiling of the tumor, the response to chemotherapy and the outcome of the patient. As control we will include sera of healthy donors of different age groups and sera of patients with other tumours, like neuroblastoma, that play a role in differential diagnosis.

⇨ **Primary goals.** The pattern of the identified antigens will contribute to answer key questions about the humoral immune response in Wilms tumor patients:

- ❖ Are Wilms tumors associated with frequent antibody response?
- ❖ Is there a complex and/or specific antibody response?
- ❖ Is this response associated with specific genetic features like gene amplifications or DNA losses?
- ❖ Do these immunogenic antigens share common features like specific sequence motives?
- ❖ Does the seroreactivity pattern allow early identification of Wilms tumors and also their histological subtypes?
- ❖ Does the seroreactivity pattern represent a prognostic marker for Wilms tumors in respect to chemotherapeutic response and / or outcome?

- ⇨ **Secondary goals.** It will also be asked for all patients with a wilms tumor how often will there be a reaction against antigen 1, how often against antigen 2 and so on. This is the question of frequency of reactivity of the different antigens in Wilms tumor patients. This frequency of antigen reactivity will be compared to sera of healthy patients and to sera of patients with other cancers. As a result a specific pattern of antigens will be found for Wilms tumor patients.

- ⇨ **Data presentation of the humoral immune response.** Positive clones identified in our primary screening of cDNA expression library will be presented as a list providing the ID of the immunogenic antigens, the frequency of the seroreactivity for each antigen both for each Wilms tumor patient and for healthy controls, and data of the immunogenicity in other cancer types.

- ⇨ **Computational analysis.** The reactivity patterns of Wilms tumor sera will be analyzed by using several statistical learning methods including linear discriminant analysis, quadratic discriminant analysis, and support vector machines. Data for computational analysis will be extracted from different databases. The computational analysis of the positive clones will be performed with antigens that are exclusively found with Wilms tumor sera or that are at least twice as frequent in sera of Wilms tumor patients as in sera of healthy donors. ➔ We will compare the antigen pattern of Wilms tumors (any histological risk/subtype) with healthy control sera, as well as each histological subtype with the healthy control group. Additionally we are going to compare the different tumor subtypes among each other to identify antigens that allow a classification of patients' sera. In the end we hope to implement a statistical learning method that allows a differentiation between Wilms tumor and normal sera, as well as a differentiation of the different subtypes.

- ⇨ **Scope.** The Scenario applies to the nephroblastoma trial SIOP 2001/GPOH. Included will be newly diagnosed patients with nephroblastoma.

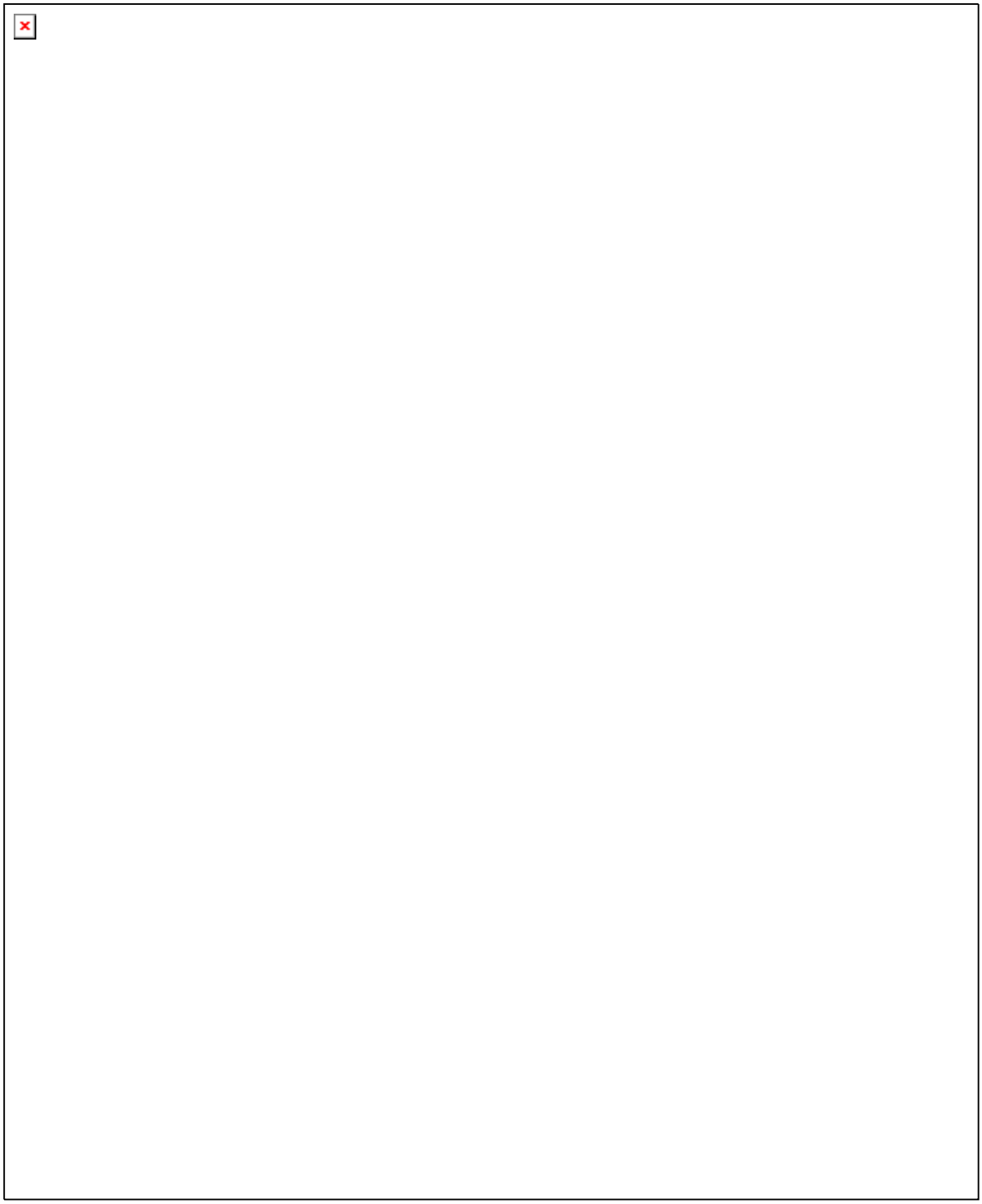
- ⇨ **Importance of the problem.** Patients with Wilms Tumour treated according to the SIOP Protocol start treatment without a histological proof of the tumour. About 1 % of patients are treated with chemotherapy having a benign lesion. (a localised area of diseased or disordered tissue.)

- ⇨ **Benefits of solving this problem.** Finding a typical pattern for nephroblastoma will help to make the correct diagnosis. This pattern will also be used as a tumor marker during follow-up. If different signatures between different histological subtypes are found, patients can be treated more individualized from the beginning according to their risk group.

II.9.2 Service Description

Serum of patients at different time points, starting with the time at diagnosis will be taken. The serum will be analyzed according to the scenario description.

A schematic description is shown in the following figure (next page). Following the schema of the scenario further explanations are given.



cDNA expression libraray. A collection of proteins, created by cDNA E. coli expression. This collection contains about 30.000 different proteins. All of these proteins are sequenced, but it is not clear if they are all existit in human beings.

[a] By performing the immunoscreening with pooled sera of patients with nephroblastoma (step 1) about (30), (40), 50, (60), proteins will be found that react with antibodies of the sera of patients. It is not known if these antigens (proteins) are expressend in the tumor. Informations about these antigens are available in the databanks listed in 3. These databases have to be used for the description of the used antigens in the experiment and are necessary for step 2.

Description of data that will be created by step two is as follow:

Data Pool of Antigens	timepoint*	antigen 1	antigen 2	antigen 3	antigen 4	antigen 5	...	...	Antigen n
Patient 1	1	+	-	-	+	+	...	...	+
Patient 1	2	+	+	-	-	-	...	...	+
Patient 1	3	+	-	+	+	+	...	...	+
Patient 1	4	+	+	-	-	-	...	...	-
Patient 2	1	-	+	-	+	+	...	...	-
Patient 2	2	+	+	-	-	+	...	...	-
Patient 2	3	-	-	+	+	+	...	...	+
Patient 2	4	+	+	-	+	-	...	...	-
Patient n	1	-	-	+	-	+	...	...	+
Patient n	2	-	+	+	-	-	...	...	+
Patient n	3	+	+	+	+	+	...	...	-
Patient n	4	-	+	-	-	+	...	...	+

* timepoint 1: at diagnosis, without treatment
timepoint 2: after preoperative chemotherapy, before surgery (not available in primarily operated patients)
timepoint 3: after surgery
timepoint 4: at the end of treatment

Coding of the histological data according to the histological subtype defined by the revised SIOP Working Classification of renal tumors of childhood (2001) (161)

	Code
A. FOR PRETREATED CASES	
I LOW RISK TUMOURS	
- *Mesoblastic nephroma*	1
- Cystic partially differentiated nephroblastoma	2
- Completely necrotic nephroblastoma	3
II INTERMEDIATE RISK TUMOURS	
- Nephroblastoma - epithelial type	4
- Nephroblastoma - stromal type	5
Nephroblastoma - mixed type	6
- Nephroblastoma - regressive type	7
- Nephroblastoma - focal anaplasia	8
III HIGH RISK TUMOURS	
- Nephroblastoma - blastemal type	9
- Nephroblastoma - diffuse anaplasia	10
- *Clear cell sarcoma of the kidney*	11
- *Rhabdoid tumour of the kidney*	12
B. FOR PRIMARY NEPHRECTOMY CASES	
I LOW RISK TUMOURS	
- *Mesoblastic nephroma*	1
- Cystic partially differentiated nephroblastoma	2
II INTERMEDIATE RISK TUMOURS	
- Nephroblastoma - epithelial type	4
- Nephroblastoma - stromal type	5
- Nephroblastoma - mixed type	6
- Nephroblastoma - blastemal type	9
- Nephroblastoma - focal anaplasia	8
III HIGH RISK TUMOURS	
- Nephroblastoma – diffuse anaplasia	10
- *Clear cell sarcoma of the kidney*	11
- *Rhabdoid tumour of the kidney*	12

- **Coding of the gene expression profiling:** Raw data of the gene expression profiles will be uploaded in the following database: http://www.ebi.ac.uk/miamexpress/**,** and can be retrieved from this database.

- **Coding the response to chemotherapy:** Response to chemotherapy can only be measured in patients receiving preoperative chemotherapy. The tumor volume will be used as response criterium. In all patients the tumor volume will be measured according to the ellipsoid formula by three dimensions (V = a * b * c * 0.526a: lenghth, b: width, c: depth, always largest diameter). Timepoints for measurements are at diagnosis (V_1: volume at diagnosis) and after preoperative chemotherapy before surgery (V_2: volume after preoperative chemotherapy). As response parameter the percentage of tumour regression will be used, calculated according to formula: V_R [%] = 100 – (V_2 * 100)/ V_1)**.** A second way for coding should be response yes or no, defined by a reduction of at least 10 % will be coded as yes, all others as no.

- **Coding of outcome:** The outcome at 2 years will be coded as complete remission, as relapse or as death. Besides that event free survival (EFS) and overall survival (OS) will be used after 2 years of follow-up. EFS and OS will be calculated according to Kaplan Meier.

[1]**Pathognomonic pattern for Wilms tumor .** The question that should be answered is:

Is the antigen pattern at diagnosis pathognomonic for Wilms tumor?

This can be answered by comparing the frequency of the different antigens found in the whole cohort of patients at timepoint 1 (diagnosis) with the frequency found in healthy persons and other malignant diseases in childhood. Most important are Clear cell sarcoma, rhabdoid tumor, oncocytoma, neuroblastoma and benign lesions of the kidney, like adenoma, and others for differential diagnostic purposes.

[2]**Pathognomonic pattern for each histological subtype of Wilms tumor.** The question that should be answered is:

Is the antigen pattern at diagnosis pathognomonic for each histological subtype of Wilms tumor?

This can be answered by comparing the frequency of the different antigens found in the whole cohort of patients with a specific histological subtype at timepoint 1 (diagnosis) with the frequency found in other histological subtypes.

[3]**Expressing genes coding for antigens.** The question that should be answered is:

Is there an expression of those genes in the tumour coding for the antigens found in step 2 of the scenario?

This question can be answered by comparing the gene expression profile of the tumour with the genes coding for the antigens (proteins) found in the scenario at timepoint 2 (after preoperative chemotherapy or before surgery). In case of primary surgery timepoint 1 will be used in this analysis. This comparison is based on the individual gene expression profile and the antigens found in the same patient. The genes coding for the antigens will be found and described by the above mentioned databases.

[4]**Pathognomonic pattern for response.** The question that should be answered is:

Is it possible to define a specific pattern of antigens that correlate with the response to preoperative chemotherapy?

To answer this question, the following data are necessary: Tumorvolume at timepoint 1 and 2 and pattern of antigens at the same timepoints. There should be correlations done

between the whole pattern of antigens and each antigen for individual patients as well as for the whole group of patients. Tumour volume should be coded in both ways as defined above.

[5] **Pathognomonic pattern for outcome.** The question that should be answered is:

Is it possible to define a pathognomonic pattern of antigens that correlates with the outcome of the patients?

To answer this question, the following data are necessary: The pattern of antigens at timepoint 1, 2, 3 and 4 and the clinical situation of the patient at 2 years after diagnosis. The clinical situation at 2 years will be coded as complete remission, as relapse or as death. A second correlation should be with EFS and OS. There should be correlations done between the whole pattern of antigens and each antigen at every timepoint for the whole group of patients.

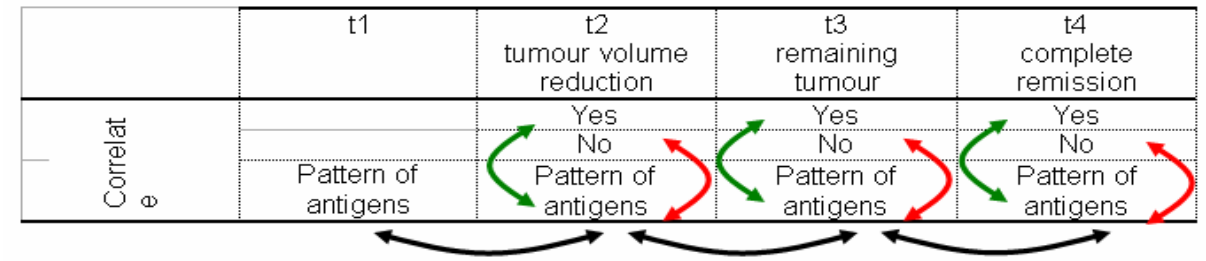
[6] **Monitoring antigen pattern over time as a marker for follow-up.** The questions to be answered are:

Does the individual pattern of antigens reflect the course of disease over time?

or

Can this antigen pattern or single antigens be used as a tumour marker?

To answer these questions, the pattern of antigens at the 4 different timepoints will be correlated with the clinical course of each patient. T1, t2 and t3 will be correlated to the tumour volume, and t4 with the remission status outcome of the patient. At timepoint t2 (after preoperative chemotherapy), there will be a tumour volume reduction or not. At timepoint t3 (after surgery), there will be remaining tumour or not. At timepoint t4 (at the end of treatment) the patient will be in complete remission or not.



⇨ **Description of Service Provider.** The ACGT platform should provide this Service.

⇨ **Description of Service Beneficiaries**

- Patients with nephroblastoma
- Molecular biologist performing the analysis
- Other researchers

II.9.3 Information Requirements

The method of the scenario for the analysis of the immunogenic tumor-associated antigens is described in the following paper:

Nicole Comtesse, Andrea Zippel, Sascha Walle, Dominik Monz, Christina Backes, Ulrike Fischer, Jens Mayer, Nicole Ludwig, Andreas Hildebrandt, Andreas Keller, Wolf-Ingo Steudel, Hans-Peter Lenhof, Eckart Meese. **Complex humoral immune response against a benign tumor: Frequent antibody response against specific antigens as diagnostic targets**. *PNAS* 102:9601-9606, 2005 (24).

The analysis of the immunogenic tumor-associated antigens will be done in the Lab of Prof. Dr. Meese at the UdS. Gene expression analysis are done in the Lab of Prof. Dr. M. Gessler in Würzburg.

Statistical analysis includes the availability of the clinical data provided by the nephroblastoma trial SIOP 2001/GPOH, and the data of both Labs. The endpoints of the statistical analysis are tables and patterns of signatures as shown for brain tumours in the above mentioned paper.

Data for computational analysis have to be extracted from the following different databases.

Information on chromosomal localization, protein function, and subcellular localization has to be retrieved from	National Center for Biotechnology Information	www.ncbi.nlm.nih.gov
	GeneCards	http://bioinfo.weizmann.ac.il_cards_index.shtml
Information on pathways has to be retrieved from	KEGG PATHWAY database	www.genome.jp_kegg_pathway.html
Information on domains has to be retrieved from	SMART database	http://smart.embl-heidelberg.de
Information about antigens found in other tumours from	SEREX database Cancer Immunome database	http://www2.licr.org/CancerImmunomeDB/
	CAP * (Cancer associated proteins) database	http://www.bioinf.uni-sb.de/CAP/
Information about autoimmunity of antigens from	the autoimmune database	http://www.wiley-vch.de/contents/jc_2040/2005/25481_s.pdf

Prediction of cleavage sites for granzyme B will be done by GRABCAS, a recently developed prediction tool for granzyme B and caspase cleavage sites that is based on experimentally determined substrate specificities. **

* Pierre Dönnes, Annette Höglund, Marc Sturm, Nicole Comtesse, Christina Backes, Eckart Meese, Oliver Kohlbacher, Hans-Peter Lenhof: **Integrative analysis of cancer-related data using CAP**. *FASEB J* 18:1465-1467, 2004 (42)

** Backes C, Kuentzer J, Lenhof HP, Comtesse N, Meese E: **GraBCas: a bioinformatics tool for score-based prediction of Caspase- and Granzyme B-cleavage sites in protein sequences**. *Nucleic Acids Research* 33: W208-W213, 2005 (3)

II.9.4 Description of the required solution in terms of the User

We choose to implement an prototyping IT service for automated statistical and computational analysis of clinical data, data from Serex analysis and data from tumor profiling of patients enrolled in the nephroblastoma trial SIOP 2001/GPOH. This IT services should be an example to perform the same analysis in other cancer and should be available to other research groups

- **Stakeholders Profile**

  Prof. Dr. Eckhart Meese, Department of Human Genetics and Molecular Biology, UdS

  Prof. Dr. Manfred Gessler, Physiologische Chemie I, Universität Würzburg

  Prof. Dr. Norbert Graf, Alexander Hoppe, UdS

- **User Profiles**

User	Responsibility	Success criteria	Deliverables
Prof. Dr. Eckhart Meese	To perform the analysis of the immunogenic tumor-associated antigens	To provide the raw data	To provide the raw data
Prof. Dr. Manfred Gessler	To perform the tumor profiling	To provide the raw data of the tumor profiling	To provide the raw data of the tumor profiling
Prof. Dr. Norbert Graf Alexander Hoppe	To collect the clinical data	To provide the clinical data	To provide the clinical data

- **Product Features.** The product should be a **Web Service** that can easily accessed by the stakeholders.

- **IT Service Benefits for Users and Stakeholders**

Stakeholder benefit	Supporting features
Automated analysis of a very complex scenario	Collecting of data, access to the different databases, statistical and computational analysis

⇨ **Assumptions and Dependencies**
- ***Constraints.*** Every researcher in an ACGT trial, who wants to perform the same analyses in other cancer types should have access.
- ***Precedence and Priority.*** None
- ***Other Product Requirements.*** None

II.10 Practical Organisation of the Trial and Study

✓ **Membership.** To participate in the study each institution should have one responsible physician. A declaration form signed by a paediatric oncologist, paediatric surgeon, radiotherapist and pathologist must be available at the study office.

✓ **Registration.** After pre-treatment investigations all patients with a renal tumour must be registered. Patients already treated for the same disease more than 3 months before the diagnostic work-up will not be registered.

✓ **Randomasied Procedure.** Randomization will take place in a period between the announcement of the definitive classification by the local pathologist and the start of treatment allowing the patient and his or her parents enough time to have a decision under consideration. All randomisation procedures will be performed by the statistical office of this study, based at the comprehensive cancer centre in Amsterdam. For France, Germany and the U.K. there will be a national co-ordinating centre to fascillitate the practical execution of the randomization. All other centres will directly contact the comprehensive cancer centre in Amsterdam.

Requests for randomisation will be accepted and issued only by the use of a randomisation form sent by fax or email. After confirmation of the randomisation form, under some

circumstances this can be permitted by telephone, the randomisation treatment allocations will be confirmed in writing to the treatment centre.

Randomisation can take place during office hours in Amsterdam (Monday to Friday from 9.00-17.00 hrs local time):

Tel: 31 - 20 - 34 62 544
Fax: 31 - 20 - 34 62 525
Email: trialbureau@ikca.nl

✓ **Ethical Considerations.** The responsible investigator will ensure that this study is conducted in agreement with the declarations of Helsinki. The outline of the randomized study will be explained and written informed consent will be obtained. The study will have to be approved by the institutional or national review board according to the legal WHO guidelines.

II.10.1 Reporing Serious Adverse Events

An Adverse Event (AE) is any untoward medical occurence or experience in a patient or clinical investigation subject which occurs during or following treatment regardless of the causal relationship. This can include any unfavourable and unintended signs (such as rash or enlarged liver), or symptoms (such as nausea or chest pain), an abnormal laboratory finding (including blood tests, x-rays or scans) or a disease temporarily associated with the treatment.

Serious Adverse Events (SAE) are defined as any undesirable experience occuring to a patient, whether or not considered related to the treatment. Adverse events which are considered as serious are those which result in:

- ⇨ death
- ⇨ a life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- ⇨ hospitalization or prolongation of hospitalization
- ⇨ severe/permanent disability
- ⇨ a congenital anomaly

Note that any death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Unexpected Serious Adverse Events are those SAE's of which the nature or severity is not consistent with information in the relevant source documents. For a medicinal product not yet approved for marketing in a country, a company's Investigator's Brochure will serve as a source document in that country.

During protocol treatment all deaths, all SAE's that are life-threatening and any *unexpected* SAE must be reported to the SIOP Nephroblastoma Trial & Study Office within 48 hours of the initial observation of the event. All details should be documented on the Serious Adverse Event and Death Report. In circumstances where it is not possible to submit a complete report an initial report may be made giving only the mandatory information. Initial reports must be followed-up by a complete report within a further 14 calendar days and sent to the SIOP Nephroblastoma Trial & Study Office. All SAE Reports must be dated and signed by the responsible investigator or one of his/her authorized staff members.

At any time after the completion of protocol treatment, *unexpected* Serious Adverse Events that are considered to be possibly related to protocol treatment and ANY death (regardless the cause) must also be reported to the SIOP Nephroblastoma Trial & Study Office using the same procedure, within 48 hours after the SAE or death was known to the investigator.

The investigator will decide whether the serious adverse event is related to the treatment (i.e. unrelated, unlikely, possible, probable, definitely and not assessable) and the decision will be recorded on the serious adverse event form. The assessment of causality is made by the investigator using the following.

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patients clinical condition, other concomitant treatments)
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patients clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

It is of utmost importance that all SAE's (including all deaths due to any cause) are reported in a timely fashion. Patients without a report of an SAE are implicity considered alive without SAE. This information will be used in monitoring the incidence of SAE's, the estimation of overall survival and monitoring of safety of experimental treatments.

II.10.2 Reporting of Adverse Events (AEs) & Severe Adverse Reactions (SARs)

II.10.2.1 Scenario description

This scenario sets out guidance on the collection, verification, presentation and decoding procedures of adverse event/reaction reports arising from clinical trials on medicinal products for human use.

All clinical trials within the European Community have to be done according to the regulations of the Directive 2001/20/EC. The responsibilities of the investigator in relation to the notification of Adverse Events (AEs) are set out in this Directive: “The investigator shall report all Serious Adverse Events (SAEs) immediately to the sponsor except for those that the protocol or investigator's brochure identifies as not requiring immediate reporting. The initial report shall be promptly followed by detailed, written reports. The initial and follow-up reports shall identify the trial subjects by unique code numbers assigned to the latter”.

Adverse events and/or laboratory abnormalities identified in the protocol as critical to the evaluation of safety must be reported to the sponsor by the investigator according to the reporting requirements within the time periods specified in the protocol. The investigator shall supply the sponsor and the Ethics Committee with any additional requested information, notably for reported deaths of a subject.

The sponsor of a clinical trial has the obligation to report all Severe Adverse Events (SAEs) and Suspected Unexpected Severe Adverse Reactions (SUSARs) to the legal authorities, the ethical committees and the participating centers.

A detailed guidance on the collection, verification and presentation of adverse event/reaction reports, together with decoding procedures for unexpected serious adverse reactions is published:
http://eudract.emea.eu.int/docs/Detailed%20guidance%20collection%20of%20adverse%20events.pdf

- *Scope.* The Scenario applies to all clinical trials on medicinal products for human use conducted within the European Community. It applies to all investigational medicinal products (IMPs) for human use, independently from their marketing authorisation status in any Member State whether or not IMPs are used under the conditions of the marketing authorisation.
- *Importance of the problem.* All suspected adverse reactions related to an IMP (the tested IMP and comparators) which occur in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting. (see also 2.1)
- *Benefits of solving this problem.* Because the investigator of each clinical trial conducted within the European Community needs to report on AEs and SARs, there are the following advantages by having one common reporting system:
  - Investigators (clinicians) have to work only with one reporting system, independent from the trial
  - Sponsor and legal authorities will get reports in a standardized way
  - EAs and SARs can easily be checked, if they are caused by the same IMP independent from the trial, making clinical trials mor esecure for patients

II.10.2.2 Service Description

- *Recording and Evaluation of Adverse Events (AEs).* Individual adverse events should be evaluated by the investigator and where indicated by the guidance in section 5, they should be reported to the sponsor for evaluation. This includes the evaluation of its seriousness and the causality between the investigational medicinal product(s) and/or concomitant therapy and the adverse event.
- The sponsor has to keep detailed records of all AEs reported to him by the investigator(s´) and to perform an evaluation with respect to seriousness, causality and expectedness. On request of a competent authority in whose territory the clinical trial is being conducted, the sponsor should submit detailed records of all adverse events which are reported to him by the relevant investigator(s´). Case report processing concerns evaluation of data in individual cases, identification of individual cases requiring specific handling, recognition and processing of alerts, and any other data processing of aggregated cases.
- *Reporting of Serious Adverse Reactions (SARs).* All suspected adverse reactions related to an IMP (the tested IMP and comparators) which occur in the concerned trial, and that are both unexpected and serious (SUSARs) are subject to expedited reporting. Additionally for IMPs that have not a marketing authorisation in any MS of the European Community, any other SUSAR associated with the IMP and as soon as the sponsor becomes aware of them are subject to expedited reporting. This includes:
  - SUSARs which occur in another trial conducted by the same sponsor either in European Community or in a third country (i.e. in non European Community countries),
  - or which are identified by spontaneous reports or a publication,
  - or which are transmitted to the sponsor by another regulatory authority.
- *Who has to report?* The investigator shall report all Serious Adverse Events (SAEs) immediately to the sponsor except for those that the protocol or investigator's brochure identifies as not requiring immediate reporting. The sponsor should report all the relevant safety information previously described to the concerned competent authorities and to the Ethics Committee concerned. The sponsor shall inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects

- *When to report?*
  - *Fatal or life-threatening SUSARs.* The competent authority and the Ethics Committee in the concerned Member States should be notified as soon as possible but no later than 7 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to the competent authority and the Ethics Committee in the concerned Member States within an additional eight calendar days.
  - *Non fatal and non life-threatening SUSARs***.** All other SUSARs and safety issues must be reported to the competent authority and the Ethics Committee in the concerned Member States as soon as possible but no later than 15 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. Further relevant follow up information should be given as soon as possible.

II.10.2.3 Other safety issues requiring expedited reporting

Other safety issues also qualify for expedited reporting where they might materially alter the current benefit-risk assessment of an IMP or that would be sufficient to consider changes in the IMP administration or in the overall conduct of the trial, for instance:

- single case reports of an expected serious adverse reactions with an unexpected outcome (e.g. : a fatal outcome),
- an increase in the rate of occurrence of an expected serious adverse reaction, which is judged to be clinically important,
- post-study SUSARs that occur after the patient has completed a clinical trial and are reported by the investigator to the sponsor,
- new event relating to the conduct of the trial or the development of the IMP likely to affect the safety of the subjects, such as :
  - a serious adverse event which could be associated with the trial procedures and which could modify the conduct of the trial,
  - a significant hazard to the subject population such as lack of efficacy of an IMP used for the treatment of a life-threatening disease,
  - a major safety finding from a newly completed animal study (such as carcinogenicity).

Where the IMP is authorised in a MS and the sponsor is the marketing authorisation holder, the reporting of SUSARs should take into account national requirements intended to manage duplication of reports in the context of the Directive 2001/83/EC, Regulation 2309/93/EC and the: 'Detailed guidance on the European database of Suspected Unexpected Serious Adverse Reactions (Eudravigilance – Clinical Trial Module)' cases.

- **Description of Service Provider.** The ACGT plattform should provide this Service.
- **Description of Service Beneficiaries**
  - Investigator (clinician) in a clinical trial of ACGT
  - Sponsor of an ACGT trial
  - competent authority in each country
  - Ethics Committee(s) of the trial
  - EMEA Database

  In accordance with national legislation, sponsors may be able to fulfil their obligation to reports SUSARs to the MS competent authority by reporting them directly to the EMEA database established under Article 11(1) of the Directive 2001/20/EC. This will avoid duplicate reporting to the EMEA database where the same trial is conducted at sites in more than one Member State and would result in more than one MS making the same report to the EMEA database.

- **Information Requirements**
  - **Minimum criteria for initial expedited reporting of SUSARs.** Information on the final description and evaluation of an adverse reaction report may not be available within the required time frames for reporting. For regulatory purposes, initial expedited reports should be submitted within the time limits as soon as the minimum following criteria are met:
    - a suspected investigational medicinal product,
    - an identifiable subject (e.g. study subject code number),
    - an adverse event assessed as serious and unexpected, and for which there is a reasonable suspected causal relationship,
    - an identifiable reporting source,
    - and, when available and applicable:
      - an unique clinical trial identification (EudraCT number or in case of non-European Community trials the sponsor's trial protocol code number)
      - an unique case identification (i.e. sponsor's case identification number)
- **Follow-up reports of SUSARs.** In case of incomplete information at the time of initial reporting, all the appropriate information for an adequate analysis of causality should be actively sought from the reporter or other available sources. The sponsor should report further relevant information after receipt as follow-up reports. In certain cases, it may be appropriate to conduct follow-up of the long-term outcome of a particular reaction.
- **How to inform the Ethics Committee?** In accordance with national legislation, the Ethics Committee concerned may only receive expedited individual reports of SUSAR that occurred in subjects who have been recruited at that Member State, provided that:
  - All SUSARs from Member States and, where applicable, from third countries are reported at least quarterly, as a line listing accompanied by a brief report by the sponsor highlighting the main points for concern. In that case, a copy should be sent to the competent authority concerned.
  - Any changes increasing the risk to subjects and any new issues that may affect adversely the safety of the subjects or the conduct of the trial should also be provided as soon as possible, but not later than fifteen days.
- **Format of the SUSARs reports.** Electronic reporting should be the expected method for expedited reporting of SUSARs to the competent authority. In that case, the format and content as defined by the Guidance1 should be adhered to. The CIOMS-I form is a widely accepted standard for expedited adverse reactions reporting. However, no matter what the form or format used, it is important that the basic information/ data elements described in annex 3, when available, be included in any expedited report. The latest version of MedDRA should be applied, using version 4.1 or later versions. Lower level terms (LLT) should be used.

Form and format of the reports about other important safety issues also qualifying for expedited reporting

Other important safety issues also qualifying for expedited reporting, should be notified by a letter under the heading of safety report. The fist page of the report should reference the EudraCT number, the title and the sponsor's trial protocol code number of the trial to which it refers and points of concern summarised in a short section.

- **SUSARs identification and management of follow-up and duplicate reports.** Each initial and follow-up SUSAR report should contain enough information to allow identification of duplicate reports. Particularly, the identification code of the patient who experienced a SUSAR must be unique in the same clinical trial whatever the number of SUSARs and the time at which they occurred. If duplicates are identified by the sponsor, the concerned competent authority and the Ethics Committee concerned shall be informed accordingly.

- **Frequency.** The competent authority and the Ethics Committee in the concerned Member States should be notified as soon as possible but no later than 7 calendar days after the sponsor has first knowledge of the minimum criteria for expedited reporting. In each case relevant follow-up information should be sought and a report completed as soon as possible. It should be communicated to the competent authority and the Ethics Committee in the concerned Member States within an additional eight calendar days. All SUSARs from Member States and, where applicable, from third countries are reported at least quarterly to the Ethics Committee, as a line listing accompanied by a brief report by the sponsor highlighting the main points for concern. In that case, a copy should be sent to the competent authority concerned.
- **Annual safety reports.** In addition to the expedited reporting, sponsors shall submit, once a year throughout the clinical trial or on request a safety report to the competent authority and the Ethics Committee of the concerned Member States, taking into account all new available safety information received during the reporting period. This global analysis should be the same for the competent authorities concerned and the Ethics Committee concerned.
- **Content of the annual safety report of a clinical trial.** The annual safety report of a clinical trial should have three parts:
  - *report on the subjects' safety in the concerned clinical trial.* The sponsor has to provide a concise safety analysis and benefit-risk evaluation for the clinical trial concerned. It should describe in a concise way, all new findings known by the sponsor related to the safety of the IMP treatments in the concerned trial and provide critical analysis of them with respect to their impact for the subjects of the concerned trial. The concept of new findings refers to information not already present in the investigator's brochure or it should be complemented with an analysis of the implications for the population of the clinical trial and should also analyse the safety profile of the tested IMP and its implication to subjects' exposure, taking into account all available safety data. When relevant, the following points should be considered:
    1. relation with dose, duration, time course of the treatment
    2. reversibility
    3. evidence of previously unidentified toxicity in the trial subjects
    4. increased frequency of toxicity
    5. overdose and its treatment
    6. interactions or other associated risks factors
    7. any specific safety issues related to special populations, such as the elderly, the children or any other at risk groups.
    8. positive and negative experiences during pregnancy or lactation
    9. abuse
    10. risks which might be associated with the investigation or diagnostic procedures of the clinical trial

    The report should also consider supporting results of non-clinical studies or other experience with the investigational medicinal product that are likely to affect the subjects' safety. It should detail the measures previously or currently proposed to minimise the risks found where appropriate. Finally, a detailed rationale must be given on whether or not it is necessary to amend the protocol, to change or update the consent form, patient information leaflet and the investigator's brochure. This report will not replace the request for protocol amendments, which will follow its own specific procedure.
  - *line listing of all suspected SARs (including all SUSARs) occurred in the concerned.* The annual report should contain a trial-specific line-listing of all reports of suspected SARs that were reported during this trial. The line listing provides key information but not necessarily all the details usually collected on individual cases. It should include each subject only once regardless of how many adverse reaction terms are reported

for the case. If there is more than one reaction, they should all be mentioned but the case should be listed under the most serious adverse reaction (sign, symptom or diagnosis) as judged by the sponsor. It is possible that the same subject may experience different adverse reactions on different occasions. Such experiences should be treated as separate reports. Under such circumstances, the same subject might then be included in a line listing more than once and the line-listings should be cross-referenced when possible. Cases should be tabulated by body system (standard system organ classification scheme). The line listing identifiable by the sponsor listing reference number or date and time of printing should include the information per case as described in annex 4.

Usually there should be one listing for each trial, but separate listings might be provided for active comparator or placebo or when appropriate and relevant for other reasons, e.g. in the case that in the same trial for different formulations, indications or routes of administration are studied.

- *aggregate summary tabulation of suspected SARs that occurred in the concerned trial.* In addition to individual cases line listings, summary tabulations of SAR terms for signs, symptoms and/or diagnoses across all patients should usually be presented to provide an overview for the trial. These tabulations ordinarily contain more terms than subjects. When the number of cases is very small, a narrative description would be more suitable. The aggregate summary tabulation should specify the number of reports :
  11. for each body system
  12. for each Adverse Drug Reaction (ADR) term
  13. for each treatment arm, if applicable (IMP, comparator or placebo, blinded treatment).

  The unexpected ADR terms should be clearly identified in the tabulation. As an example, the table in annex 5 can be used. When the sponsor conducts several clinical trials with the same tested IMP, a single annual safety report referring to several trials could be acceptable. In that case:
  1. a concise global analysis on the safety profile of the tested IMP taking into account all new findings related to the safety of the tested IMP in the concerned clinical trials and an analysis of the implications of the findings for the population included in each clinical trial covered by the report
  2. and the annual safety report relating to each clinical trial concerned.

- **Volume.** The volume is specified by the form and the content of the report.
- **Duration /Interval.** The reporting time frame for annual reports starts with the date of the first authorisation of the concerned clinical trial by a competent authority in any Member State. This date is designated as the cut off for data to be included in the annual safety report. The sponsor should submit annual reports within 60 days of the data lock point. However, if a sponsor conducts several clinical trials with the same tested investigational medicinal product in any Member State, he should prepare only one safety report covering the information necessary for all those trials, the reporting period starts with the date of the authorisation for the first of these trials by the competent authority in any Member State and ends after close of the last trial in any MS. If the sponsor is the marketing authorisation holder (MAH) of the tested IMP, the reporting period should be aligned with the International Birth Date. However, Annual Safety Report and Periodic Safety Update Report (PSUR) must be stand-alone documents. If the IMP is granted a marketing authorisation for the first time in any MS while it is being tested in a clinical trial, the reporting time frame for the IMP would change from the first date of authorisation of a clinical trial in a MS to the international birth date. In the case of short term trials (less than 6 months), the safety report may be notified within 90 days of the end of trial together with the notification of the end of the trial according to Directive 2001/20/EC article 10 c). This report should contain at least line listings, if appropriate aggregate summary tabulations and a statement of the patients safety.

- **How to inform the investigators?** The sponsor shall inform all investigators concerned on findings that could adversely affect the safety of study subjects. If appropriate, the information can be aggregated in a line listing of SUSARs in periods as warranted by the nature of the clinical development project and the volume of SUSARs generated. This line listing should be accompanied by a concise summary of the evolving safety profile of the investigational medicinal product.
  In the case of blinded trials the line listing should present data on all SUSARs, regardless of the medication administered (e.g. active/placebo), thereby when possible and appropriate, the blind would be maintained and the risk of inadvertently informing the investigators with regard to the identity of the medication would be avoided.

  If a significant safety issue is identified, either upon receipt of an individual case report or upon review of aggregate data, the sponsor should issue as soon as possible a communication to all investigators. A safety issue that impacts upon the course of the clinical study or development project, including suspension of the study programme or safety-related amendments to study protocols should also be reported to the investigators.

Reporting of safety issues following completion of the clinical trial in European Community

After termination of the clinical trial, any unexpected safety issue that changes the risks benefit analysis and is likely to have an impact on the subjects who have participated in it, should be reported as soon as possible to the competent authority(ies) concerned together with proposed actions.

- **Info source.**
  http://eudract.emea.eu.int/docs/Detailed%20guidance%20collection%20of%20adverse%20events.pdf
- **Location of information source.** Clinical database of the trial
- **Description of information.** See Annex 2, 3, 4, 5
- **Access/Security.** Pseudonymisation of personal data is required
- **Processing of information.** The principal investigator sends the information of an AE or an SAR to the Sponsor of the trial. The sponsor has to send the information to the ethical committee, the legal authorities and the participating centers. The procesing of the information is time dependend. See description above.
- **Presentation of processed information.** See Annex 3, 4, 5 and the sections before.
- **Description of the required solution in terms of the User.** A prototyping IT service has to be developed to distribute the data of AEs and SARs to support the reporting of AEs and SARs in clinical trials. The IT service has to use a service for pseudonymization of personal data. The principal investigator of a clinical trial should use this service to provide the Sponsor of the trial with the informations of AEs and/or SARs in a timely manner. The Sponsor should use this service to send the data to the legal authorities, ethical committees and the participating centers. The service should automatically generate all reports including annual reports according to the obligations defined by the Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use (April 2004).
- **Stakeholders Profile.** The following stakeholders are involved in this service:
  - Principal investigator
  - Sponsor
  - Participating center in the trial
  - Ethical committees
  - Legal authorities
  - Eudract Database

- **User Profiles**

User	Responsibility	Success criteria	Deliverables
Principal Investigator	To send AEs and/or SARs to the Sponsor	Timely income of the standardized report by the sponsor	Standardized report
Sponsor	To send the received reports of the principal investigator to the participating centers, the ethical committee and the legal authorities To send all other reports as described above, including the annual report	Timely income of the standardized report by participating centers, the ethical committees, the legal authorities and in the EudraCT Database	Standardized report
Participating centers in the trial	To receive all reports as described above	Income of the reports	
Ethical Committee	To receive all reports as described above	Income of the reports	
Legal authorities	To receive all reports as described above	Income of the reports	
EudraCT Database	To receive all reports as described above	Income of the reports	

- **Product Features.** The product should be a Web Service that can easily accessed by the stakeholders.

- **IT Service Benefits for Users and Stakeholders**

Stakeholder benefit	Supporting features
Easier work for Investigators (clinicians)	only one reporting system, independent from the trial
Less work for Sponsor and legal authorities	They will get reports in a standardized way via online access
Improved Patient security	Reports are standardized and in the same manner independent from the trial

- **Assumptions and Dependencies.** If the Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use (April 2004) will change the Service have to be adopted.

- **Constraints.** Every investigator (clinician, participating in an ACGT trial), the principal investigator of the ACGT trial, the sponsor of the ACGT trial should have access to the service.

- **Precedence and Priority.** All features are necessary.

- **Other Product Requirements.** See Annex 2, 3, 4, 5. In addition access to the EudraCT database has to be done.

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Annex 1: Comments on definitions and abbreviations

Adverse event (AE): any untoward medical occurrence in a patient or clinical trial subjectadministered a medicinal product and which does not necessarily have a causal relationship with this treatment. Comment: An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product, whether or not considered related to the investigational medicinal product

Adverse reaction of an investigational medicinal product (AR): all untoward and unintended responses to an investigational medicinal product related to any dose administered. Comment: All adverse events judged by either the reporting investigator or the sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected adverse reaction: an adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product). Comments: When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.

Severity: The term “severe” is often used to describe the intensity (severity) of a specific event. This is not the same as “serious,” which is based on patient/event outcome or action criteria.

Serious adverse event or serious adverse reaction: any untoward medical occurrence or effect that at any dose:

- results in death,
- is life-threatening
- requires hospitalisation or prolongation of existing inpatients´ hospitalisation,
- results in persistent or significant disability or incapacity,
- is a congenital anomaly or birth defect.

Comments:

- Life-threatening in the definition of a serious adverse event or serious adverse reaction refers to an event in which the subject was at risk of death at the time of event; it does not refer to an event which hypothetically might have caused death if it were more severe.
- Medical judgement should be exercised in deciding whether an adverse event/ reaction is serious in other situations. Important adverse events/ reactions that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Concerned Member State: Member State in whose territory a clinical trial with the investigational product is being performed.

Ethics Committee Concerned: Ethics Committee that gave the favourable opinion for a clinical trial on the investigational product in a Member State according to Art. 7 of the Directive 2001/20/EC.

Investigators Concerned: Investigators, which are actively involved in running clinical trials on the tested investigational medicinal product

Data Lock-Point (cut-off date): The date designated as the cut off date for data to be included in a annual safety report

International Birth Date (IBD): The date of the first marketing authorisation for a medicinal product granted to the marketing authorisation holder (MAH) in any country in the world.

Periodic Safety Update Report (PSUR) for a medicinal product with a marketing authorisation: All records of adverse reactions shall be submitted to the competent authorities in form of a periodic safety update report, either immediately upon request or periodically as follows: six monthly for the first two years after authorisation, annually for the subsequent two years, and at the time of the first renewal. Thereafter the periodic safety update report shall be submitted at five-yearly intervals together with the application for renewal of the authorisation. The periodic safety update report shall include a scientific evaluation of the benefit and risks afforded by the medicinal products.

Annex 2: Member States' Contact points for Reporting

The Member States' contact points for reports of adverse reactions occurring in clinical trials on human medicinal products are as follows:

Member state	Contact point
Belgium	Federal Public Service Health, Food Chain Safety and Enviroment Directorate-General Medicinal Products Unit IX – Clinical trials Bischhoffsheim 33, 1st floor 1000 Brussels, Belgium Phone:+ 32 (0) 2 227 55 77 Fax: + 32 (0) 2 227 55 31
Denmark	The Danish Medicines Agency Clinical Trials, Inspection and Enforcement Division Axel Heides Gade 1 DK-2300 Copenhagen S Phone: + 45 44 88 95 95 Fax: + 45 44 88 93 14 www.dkma.dk
France	Agence Francaise de Sécurité Sanitaire des Produits de Santé (AFSSAPS) DEMEB/Unité Essais Cliniques 143/147, Boulvard Anatole France 93285 Saint-Denis Cedex Phone: +33-1-55-87-36-43 Fax: +33-1-55-87-36-42
Germany	Federal Institute for Drugs and Medicinal Devices Kurt-Georg-Kiesinger-Allee 3 D-53175 Bonn Phone +49-228-207/4320 Fax: +49-228-207 Paul-Ehrlich-Institut Paul-Ehrlich-Str. 51-59 D-63225 Langen Phone: +49 6103-77-1010/1011 Fax: +49 6103-77-1263 Home page: www.pei.de e-mail: kelbr@pei.de
Greece	National Organization for Medicines (EOF) Division of Pharmaceutical Studies and Research 284 Mesogeion Avenue 15562 Athens Greece Tel + 30 210 6507200 Fax + 30 210 6549585 Home page www.eof.gr
Italy	Ministry of Health General Directorate for Drug and Medicinal Viale Civiltà Romana, 7 00 144 ROMA Phone : +39-06 5994 3483

	Fax : + 39-06 5994 3227
Ireland	Drug Safety Associate, Pharmacovigilance Unit, Irish Medicines Board; Earlsfort Centre, Earlsfort Terrace, Dublin 2, Ireland Phone: + 353-1-676 4971 Fax: + 353-1-676 2517 Home page: www.imb.ie
Luxembourg	Direction de la Santé Division de la phamrmacie et des Médicaments Villa Lowigny Allée Marconi L-2120 Luxembourg Tel: +352 478 55 93/55 90 Fax: +352 26 20 01 40/47
Netherlands	College ter Beoordeling van Geneesmiddelen/Medicines Evaluation Board PO Box 16229 2500 BE Den Haag Phone: +31 70 3406700 Fax. +31 70 3406737
Portugal	INFARMED, Departamento de Farnacovigilancia, Sector de Reaccoes Adversas a Medicamentos Parque da Saude de Lisboa Av. Do Brasil, 53 1749-004 Lisboa, Portugal Phone: +351 21 7987 100/7142 Fax: + 351 21 7987 100 Home page: www.infarmed.pt
Spain	Agencia Espanola de Medicamentos y Productos Sanitarios Division de Farmacologia y Evaluacion Clinica C/ Alcalá, 5628071 Madrid Fax: +34 91 822 5161 ▪ When the investigational medicinal product is marketed in Spain, and used under the terms of market authorisation: Division de Farmacoepodemiologia y Farmacovigilancia Paseo del Prado 18-20 28014 Madrid Fax. +34 91 596 78 91
Sweden	Pharmacovigilance Unit Medicinal Products Agency P.O. Box 26 S-751 03 Uppsala, Sweden Phone: +46 18 17 56 00 Fax: +46 18 54 85 666 e-mail: registratorpa.se home page: www.mpa.se
United Kingdom	MHRA Clinical Trials Unit Market Towers, 12th Floor

	1 Nine Elms Lane London SW8 5 NQ Phone: +44 (0) 207 084 2327 FAX: +44 (0) 207 084 2443 e-mail: salma.syed@mhra.gsi.gov.uk
Cyprus	The Registrar Drugs Council PHARMACEUTICAL SERVICES, MINISTRY OF HEALTH 1475 LEFKOSIA, CYPRUS Tel.: +357-22-407-132 Fax: +357-22-407-149
Czech Republic	State Institute for Drug Control – Branch of Clinical Trials and Pharmacovigilance Šrobárova 48 100 41 Praha 10 Fax: +420 272 185 816 Phone: +420 272 185 817 klin.sekret@sukl.cz;
Estonia	Katrin Kiisk State Agency of Medicines 19 Ravila Street 50411 Tartu Estonia Fax: + 372 737 4142 e-mail: katrin.kiisk@sam.ee
Latvia	Janis Ozolins, Head of the Board of State Agency of Medicines, 15 Jersikas street, Riga, LV 1003 Phone: 371-7078400 Fax: 371-7078428 e-mail address: info@vza.gov.lv
Norway	Norwegian Medicines Agency Section for clinical trials Sven Oftedalsvei 6 NO-0950 OSLO NORWAY Telephone: (+47) 22 89 77 00 Telefax: (+47) 22 89 77 99 Internet: www.noma.no E-mail: klut@noma.no

Annex 3: Data Elements for SUSAR report

1. Clinical trial identification:

- Clinical trial identification (EudraCT number, if applicable or the sponsor's trial protocol number),

2. Subject's details :

- Sponsor's subject identification number5,
- Initials, if applicable,
- Gender,
- Age and/or date of birth,
- Weight,
- Height,

3. Suspected investigational medicinal product(s) :

- Name of the IMP or brand name as reported,
- International non-proprietary name (INN),
- Batch number,
- Indication(s) for which suspect investigational medicinal product was prescribed or tested,
- Dosage form and strength,
- Daily dose and regimen (specify units e.g. mg, ml, mg/kg),
- Route of administration,
- Starting date and time of day,
- Stopping date and time, or duration of treatment
    - Unblinding : yes/no/not applicable ; results1:
    - Investigator's causality assessment
    - Sponsor's causality assessment

  Comments, if relevant (e.g. causality assessment if the sponsor disagrees with the reporter; concomitant medications suspected to play a role in the reactions directly or by interaction; indication treated with suspect drug(s).

4. Other treatment(s) :

- For concomitant medicinal products (including non prescription/OTC medicinal products) and non-medicinal product therapies, provide the same information as listed above for the suspected investigational medicinal product.

5. Details of suspected Adverse Drug Reaction (s) :

- Full description of reaction (s) including body site and severity, as well as the criterion (or criteria) for regarding the report as serious should be given. In addition to a description of the reported signs and symptoms, whenever possible attempts should be made to establish a specific diagnosis for the reaction.
- Reaction(s) in MedDRA terminology1 (lowest level term)6
- Start date (and time) of onset of the reaction,
- Stop date (and time) or duration of the reaction,
- De-challenge and re-challenge information,
- Setting (e.g. hospital, out-patient clinic, home, nursing home),
- Outcome : information on recovery and any sequelae; what specific tests and/or treatment may have been required and their results ; for a fatal outcome, cause of death and a comment on its possible relationship to the suspected reaction should be provided. Any autopsy or other post-mortem findings (including a coroner's report) should also be provided when available.
- Other information : anything relevant to facilitate assessment of the case, such as medical history including allergy, drug or alcohol abuse ; family history ; findings from special investigations.

6. Details on reporter of event/suspected ADR :

- name,
- address,
- telephone number,
- profession (speciality)

7. Administrative and Sponsor details:

- Date of this report
- Source of report: from a clinical trial (provide details if not in Eudract1, from the literature (provide copy), spontaneous, other,
- Date event report was first received by sponsor,
- Country in which reaction occurred,
- Type of report filed to authorities : initial or follow-up (first, second, etc),
- Name and address of sponsor/manufacturer/company,
- Name, address, telephone number and fax number of contact person in reporting sponsor,
- identifying regulatory code or number for marketing authorisation dossier or clinical investigation process for the suspected product (for example IND number, NDA number)
- Case reference number (sponsor's/manufacturer's identification number for the case) (this number must be the same for the initial and follow-up reports on the same case).

Annex 4: Content of line listing

The line listing identifiable by the sponsor listing reference number or date and time of printing should include the following information per case

1. clinical trial identification,
2. Study subjects identification number in the trial
3. case reference number (Case-ID-Number) in the sponsor's safety database for medicinal products
4. country in which case occurred
5. age and sex of trial subject
6. daily dose of investigational medicinal product, (and, when relevant, dosage form and route of administration)
7. date of onset of the adverse reaction. If not available, best estimate of time to onset from therapy initiation. For an ADR known to occur after cessation of therapy, estimate of time lag if possible.
8. dates of treatment. (if not available, best estimate of treatment duration.)
9. adverse reaction : description of reaction as reported, and when necessary as interpreted by the sponsor ; where medically appropriate, signs and symptoms can be lumped into diagnoses. MedDRA should be used.
10. patient's outcome (e.g. resolved, fatal, improved, sequelae, unknown). This field should indicate the consequences of the reaction(s) for the patient, using the worst of the different outcomes for multiple reactions
11. comments, if relevant (e.g. causality assessment if the sponsor disagrees with the reporter; concomitant medications suspected to play a role in the reactions directly or by interaction; indication treated with suspect drug(s); dechallenge / rechallenge results if available)
12. unblinding results in the case of unblinded SUSARs expectedness at the time of the occurrence of the suspected SARs, assessed with the reference document (i.e. investigator's brochure) in force at the beginning of the period covered by the report.

Annex 5: Example for an Aggregate Summary Tabulation

Number of reports by terms (signs, symptoms and diagnoses) for the trial n° : (An * indicates an example of a SUSAR)

Body system	Verum	Placebo	Blinded
CNS			
Hallucinations *	**2**	**2**	**0**
Confusion*	**1**	**1**	**0**
Sub-total	**3**	**3**	**0**
COR			
…*			
Sub-total			
…			
Total			

Annex 6: Forms, Reporting, Ethics

Forms (trial and protocol patients)

F1: Registration. Should be sent immediately to the trial and study office. The office will send to the centre the registration number alloted to the patients.

F2: Pre-operative chemotherapy. Should be sent at the end of the pre-operative chemotherapy treatment.

F3: Operative findings. Should be completed as soon as possible after finishing the operation and should be sent with a copy of the original surgical report immediately to the trial and study office. One copy of the original report should be sent to the local pathologist with the surgical specimens.

F3b: Nephrectomy-related complications checklist. Should be filled in by the attending surgeon and transferred to the oncologist-in-charge, who will have to review this checklist 1 year after surgery in view of eventual delayed postoperative complications. Then the form should be sent to the Amsterdam office.

F4: Histopathological report. For all cases the stage and the histological diagnosis is based on the local pathologists observation. For those countries with a national coordinator all slides must be received on a short term by the national-coordinating pathologist. If no national coordinator is appointed the slides must be sent to the chairman of the panel of pathologists.

At the end the slides of all patients should be discussed by the panel of pathologists.

→ Since postoperative treatment is completely based on a correct stage and histological classification it is of the utmost importance to consult an experienced pathologist in case of doubt on a short term.

Each pathologist is responsible for communicating his/her conclusion to the referring pathologist and to the data centre.

F5: Randomisation Form

F6: Radiotherapy report. This form has to be used in patients that need irradiation. The form has to filled by the local radiotherapist, who is responsible for the correct radiotherapy.

F7: Post-operative chemotherapy. Should be send at the end of the treatment

F8: SAE report. Should be used immediately after an SAE

F9: Follow-up report. Should be used at least once a year after the end of treatment and always immediately in case of an event.

IDMC (Independent Data Monitoring Committee)

The IDMC shall review the outcome data for the SIOP 2001 trials when the protocol- specified number of events or patients has been reached.

The IDMC shall review the trials with consideration of the objectives, scientific impact of the findings, and patient safety.

The IDMC shall review all modifications of the trials protocol involving a change in the accrual goals or other major changes.

The IDMC shall make recommendations in a report to the SIOP 2001 Core Committee. Recommendations will be made based on a consensus of the Committee members. The report should be completed within one month of the IDMC meeting.

The Core Committee is responsible for dissemination of results. If a trial is stopped based on an IDMC recommendation at an interim analysis, the IDMC must review the paper prior to submission to assure proper reporting of the recommendation.

Unblinded results should be presented to the IDMC during accrual.

In a trial that goes up to the planned accrual, the responsibility of the IDMC ends after the last patient enrolled has completed the planned therapy. At that time, the results are released to the Core Committee.

The IDMC will continue to monitor long-term outcome. The IDMC will also review interim efficacy reports of closed trials if requested to do so by the Core Committee.

The Chair of the IDMC will be responsible for reviewing ongoing trials reported by other major cooperative groups and for advancements in clinical recommendations in general in similar populations to ensure that ongoing trials reflect at least currently accepted "standard therapy". The Chair will recommend major changes to the Core Committee if he/she feels that patient participation in an ongoing trial is contrary to good clinical standards.

The Core Committee should inform the Chair of the IDMC if any unusual patient side-effects are encountered. This contact should be made as soon as clarified and should not wait for the next scheduled report.

Ethics

Declaration of Helsinki

The Study and Trial will be done in accordance to the last revision of the declaration of Helsinki (2000 Edinburgh,Scottland):

The World Medical Association Declaration of Helsinki

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th World Medical Association (WMA) General Assembly, Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; the 35th WMA General Assembly, Venice, Italy, October 1983; the 41st WMA General Assembly, Hong Kong, September 1989; the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996; and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000.

A. Introduction

1. The World Medical Association has developed the *Declaration of Helsinki* as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.

2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.

3. The *Declaration of Geneva* of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the *International Code of Medical Ethics* declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.

6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the aetiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.

7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.

8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will not benefit personally from the research and for those for whom the research is combined with care.

9. Research investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. Basic Principles For All Medical Research

10. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.

11. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.

12. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

13. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.

14. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.

15. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.

16. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.

17. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.

18. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.

19. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.

20. The subjects must be volunteers and informed participants in the research project.

21. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.

22. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

23. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

24. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

25. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

26. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

27. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. Additional Principles for Medical Research Combined with Medical Care

28. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

29. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

To further clarify the WMA position on the use of placebo controlled trials, the WMA Council issued, during October 2001, a Note of Clarification on Article 29:

The WMA is concerned that paragraph 29 of the revised *Declaration of Helsinki* (October 2000) has led to diverse interpretations and possible confusion. It hereby reaffirms its position that extreme care must be taken in making use of a placebo-controlled trial and that in general this methodology should only be used in the absence of existing proven therapy. However, a placebo-controlled trial may be ethically acceptable, even if proven therapy is available, under the following circumstances:

- Where for compelling and scientifically sound methodological reasons its use is ecessary to determine the efficacy or safety of a prophylactic, diagnostic or herapeutic method; or
- here a prophylactic, diagnostic or therapeutic method is being investigated for a inor condition and the patients who receive placebo will not be subject to any dditional risk of serious or irreversible harm.

All other provisions of the Declaration of Helsinki must be adhered to, especially the need for appropriate ethical and scientific review.

30. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.

31. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.

32. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

World Medical Association
Edinburgh, Scotland
October 2000

Ethical Committee

- ⇨ Study protocol, Patient information and informed consent for SIOP 2001 have passed the Ethical committee (Ärztekammer des Saarlandes, 30.09.2002). The ethical committee will be informed by the the principal investigator about every change in the protocol. He will report on SUSARs and SAEs.
- ⇨ The ethical committee will receive the amendment regarding the Scenario: “humoral response against nephroblastoma antigens“ for evaluation.

Data Transfer, storage and security

- ⇨ The patient or his legal guardian will be informed about the anonymous or pseudonymized storage of personalized data. The data will be provided for scientific purposes. The patient has the right to get information about his stored data. He has to be informed about the distribution of the data.
- ⇨ The informed consent for data storage and distribution has to be done separately to the informed consent for participation in the trial.

Informed Consent

- ⇨ Every patient or his legal guardian has to give his informed consent for participation in the trial. Patient, and legal guardian must have enough time to decide to take part. Open questions have to be clearly answered before signing the informed consent.
- ⇨ The informed consent has to be signed by the patient, (if he is old enough), or his legal guardian and the treating physician.
- ⇨ A Form of the informed consent is provided in chapter: 18.2. One exemplar has to be given to the patient or the legal guardian, the other one has to be retained by the treating physician.
- ⇨ Regarding the treatment (chemotherapy, surgery, irradiation) a separate informed consent has to be signed. This has to be done by the specific physician.
- ⇨ For biomaterial a separate informed consent has to be signed

Forms

All forms have to be provided in the language of the patient. The content has to be easily understandable for the patient or his legal guardian. (see chapter 18 with the forms for Germany)

- **Master Forms**

Documentation of Patient Education

Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH

Patient : .....
(Name)(Vorname) (geb.)

Aufklärungsgespräch am : .....200....

Gesprächspartner :.....

Sorgeberechtigte :.....
(Name, Vorname)
Patient: .....
(Name, Vorname)
Arzt: .....
(Name, Vorname, Funktion)
Zeuge: .....
(Name, Vorname, Funktion)

Die Aufklärung erstreckte sich auf:

↓ Diagnose ↓Krankheitsverlauf und Prognose ohne geeignete Therapie

↓ erwartete Prognose mit Therapie SIOP 2001 / GPOH

↓ Prognose mit alternativen Therapien (z.B. SIOP 93-01 / GPOH)

↓ Chemotherapie - Wirkung

(Tumorverkleinerung, Vernichtung von Metastasen, Mikrometastasen, Kombinations-chemotherapie zur Vermeidung von Resistenzen)

↓ Chemotherapie - Nebenwirkungen

(Übelkeit, Erbrechen, vorübergehender Haarausfall, Auswirkungen auf Knochenmark und Blutbild, erhöhte Infektionsneigung, mögliche Organschäden z.B. auf Herz, Leber, Niere, Darm, evtl. Auswirkungen auf Fertilität, Risiko einer späteren Entstehung von Tumoren, Gewebeschaden bei Fehlinfusion)

↓ Operation - Bedeutung

(möglichst vollständige Entfernung des Tumors, Gewinnung von Gewebe zur mikroskopischen Untersuchung und Molekulargenetik)

↓ Operation - Nebenwirkungen

operatives Risiko wird durch den Operateur getrennt erläutert

↓ Strahlentherapie - Wirkung

(Devitalisierung noch zurückgebliebener Tumorzellen)

↓ Strahlentherapie - Nebenwirkungen

(Wachstumsstörungen im Bestrahlungsareal bei Skelett und Weichteilen, Lungenfunktionsstörung bei Lungenbestrahlung)

Studienordnung

↓ Sinn der Studie

Vermeidung von kardialen Spätfolgen bei Patienten im Stadium II und III mit einem unilateralen Tumor mit intermediärer Malignität durch randomisierte Prüfung der Frage, ob auf die Gabe von Anthracyclinen verzichtet werden kann.

↓ Probleme der präoperativen Chemotherapie ohne histologisch gesicherte Diagnose.

↓ Verminderung des Risikos der Tumorruptur.

↓ Erniedrigung des für die weitere Behandlung entscheidenden Tumorstadiums zum Zeitpunkt der Operation.

↓ Sinn der Randomisation der postoperativen Chemotherapie bei Patienten mit dem histologischen Befund der intermediären Malignität, die sich im klinischen Stadium II oder III befinden und ein Tumorvolumen unter 500 ml zum Zeitpunkt der Operation aufweisen.

↓Zuordnung der Patienten nach Zufallsgesichtspunkten zu den Therapiezweigen mit oder ohne Anthracycline in der postoperativen Chemotherapie mit ACT-D und VCR.

↓ Blutentnahme zur Virusdiagnostik auf HIV, EBV, Hepatitis-A, -B, -C, Zytomegalie.

↓ Möglichkeit, die Einwilligung zur Studientherapie und/oder zur Randomisation zu verweigern ohne Nachteile für Patient und Sorgeberechtigten.

Über die nicht angekreuzten Punkte wurde *nicht gesprochen*.

Grund: ↓ Patientenverzicht

↓ Patientengefährdung

↓ Verzicht der Sorgeberechtigten

Entscheidung: ↓ Studienteilnahme ↓ ja ↓ nein

↓ Randomisation ↓ ja

↓ Wahlentscheidung ↓ mit Anthracyclin

↓ ohne Anthracyclin

Aufklärender Arzt:

.....

(Unterschrift, Datum)

Zeuge:

.....

(Unterschrift, Datum)

Information and consent for participation

Patienteninformation zur Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH und Einverständniserklärung zur Studienteilnahme

Betr.:Behandlung eines Nephroblastoms

bei : .....geb.: .....

Ich bestätige hiermit, dass ich heute durch den unten aufgeführten Arzt ausführlich über die bei mir bzw. bei meinem o.g. Kind bestehende Erkrankung und deren natürlichen Verlauf und Prognose ohne Behandlung unterrichtet wurde.

Zur Sicherung der Diagnose und im Verlauf der Erkrankung sind bildgebende Untersuchungen (Ultraschall, Kernspintomographie oder Computertomographie), Blutuntersuchungen und Funktionstests verschiedener Organe (Niere, Herz, Gehör) notwendig. Diese Untersuchungen reduzieren die Gefahr der Fehldiagnose und dienen dem frühzeitigen Erkennen von möglichen Gefahren durch den Tumor oder die notwendige Behandlung. Auf die Risiken von Kontrastmitteluntersuchungen bei der Bildgebung wurde ich hingewiesen.

Über mögliche Therapien der Erkrankung wurde ich ausreichend informiert. Die Behandlung soll im Rahmen der Wilms-Tumor-Therapieoptimierungsstudie SIOP 2001 / GPOH entsprechend einem Plan (Protokoll) erfolgen, nach dem über 100 deutsche und europäische Kinderkliniken vorgehen. Die Gesamtbehandlung besteht aus einer der Operation vorgeschalteten Medikamententherapie (präoperative Chemotherapie) über 4 Wochen, bzw. 6 Wochen bei vorliegenden Metastasen zur Tumorverkleinerung. Im Anschluss an die Vortherapie erfolgt die operative Entfernung des Tumors und eventuell von Metastasen.

Je nach Befund bei der Operation und dem Ausmaß, wie weit dieser Tumor entfernt werden konnte, schließt sich nach der Operation nochmals eine Medikamententherapie an (postoperative Chemotherapie).

War der Tumor ganz auf die Niere begrenzt und konnte bei der Operation der gesamte Tumor vollständig im Gesunden entfernt werden, liegt ein Stadium I der Erkrankung vor. In diesem Stadium und bei gleichzeitiger feingeweblich festgestellter mittlerer Bösartigkeit (intermediäre Malignität) des Tumors erhalten die Patienten nur einen weiteren Block Chemotherapie, wenn das Tumorvolumen zum Zeitpunkt der Operation kleiner als 500 ml war. In höheren Tumorstadien (Stadium II und III, nicht bei Patienten mit Metastasen),

mittlerer Bösartigkeit (intermediäre Malignität) und kleinem Tumorvolumen vor Operation (< 500 ml) wird die bislang routinemäßige Gabe eines Medikamentes (Doxorubicin) auf Wirksamkeit überprüft. Diese Prüfung ist sinnvoll, da dieses Medikament bei geheilten Patienten zu Spätfolgen am Herzen führen kann (Einschränkung der Herzmuskelleistung). Die Zuordnung, ob dieses Medikament gegeben wird, wird zufällig getroffen (Randomisation). Auf diesem Weg soll herausgefunden werden, ob der Verzicht auf dieses Medikament nach der Operation und damit Verminderung der Nebenwirkungen am Herzen zur gleichen Heilungsrate führt wie, wenn dieses Medikament verabreicht wird.

Konnte bei der Operation nicht das ganze Tumorgewebe entfernt werden, muss zusätzlich zur Chemotherapie eine lokale Bestrahlung erfolgen. Im Falle von Fernmetastasen ist auch eine operative Entfernung dieser Metastasen zu erwägen. Bei hoher Bösartigkeit (hohe Malignität) muss eine intensive Chemotherapie mit 4 Medikamenten gegeben werden.

Ein Schema der Therapie, die durchgeführt wird, habe ich erhalten.

In Abhängigkeit des Tumorstadiums und der Histologie werden folgende zytostatische Medikamente eingesetzt: Vincristin, Actinomycin-D, Doxorubicin, Carboplatin, Etoposid, Cyclophosphamid. Auf die Nebenwirkungen und die möglichen Spätfolgen der Chemotherapie wurde ich ausführlich hingewiesen. Dabei wurde u.a. besprochen:

Erbrechen, vorübergehender Haarausfall, Auswirkungen auf Knochenmark und Blutbild, erhöhte Infektionsneigung, evtl. Auswirkungen auf die Fertilität (Fruchtbarkeit), Risiko einer späteren Entstehung von Tumoren, örtliche Gewebsschädigung bei Fehlinfusion, verminderte Herzmuskelleistung bei Doxorubicintherapie, Störungen der Leber-, Nieren- und Darmfunktion, Innenohrschwerhörigkeit bei Carboplatingabe.

Über Risiken und Probleme der Operation sowie über die Nebenwirkungen und möglichen Spätfolgen der Bestrahlung werde ich noch ausführlich durch den Operateur bzw. den Strahlentherapeuten informiert.

Die Dauer der Behandlung richtet sich nach dem Stadium der Erkrankung und der Histologie und liegt nach Tumoroperation bei maximal 34 Wochen. Da die meisten Rezidive in den ersten beiden Jahren nach Therapieende auftreten, sind regelmäßige Untersuchunegn mindestens über diesen Zeitraum notwendig. Patienten, die Doxorubicin erhalten haben, können auch noch nach Jahren eine Einschränkung der Herzfunktion erleiden. Sie müssen bis zu 10 Jahren nach Therapieende diesbezüglich nachuntersucht werden.

Mir wurde mitgeteilt, dass für die Therapieoptimierungsstudie SIOP 2001/GPOH ein positives Votum der Ethikkommission der Ärztekammer des Saarlandes vorliegt.

Ich erkläre mich damit einverstanden, dass nach dem Behandlungsplan der Nephroblastom-Therapieoptimierungsstudie SIOP 2001 / GPOH, der mir im einzelnen erläutert wurde, vorgegangen wird. Bezüglich der Therapie wurde ich auf folgende Punkte aufmerksam gemacht:

O Probleme der präoperativen Chemotherapie ohne histologisch gesicherte Diagnose.

O Verminderung des Risikos der Tumorruptur durch eine präoperative Chemotherapie.

O Erniedrigung des für die weitere Behandlung entscheidenden Tumorstadiums zum Zeitpunkt der Operation.

O Sinn der Randomisation der postoperativen Chemotherapie im klinischen Stadium II und III bei histologischem Befund eines Nephroblastoms von mittlerer Bösartigkeit (intermediär Malignität) und kleinem Tumorvolumen (< 500 ml)

O Die Zuordnung der Patienten nach Zufallsgesichtspunkten zu den Therapiezweigen mit oder ohne Doxorubicin.

Zu Beginn und im Verlauf der Behandlung werden bei allen Kindern Blutuntersuchungen durchgeführt u.a. zum Ausschluss einer infektiösen Gelbsucht, einer Zytomegalie-Virusinfektion, einer Epstein-Barr-Virusinfektion und einer Infektion durch das HIV-Virus. Diese Infektionen könnten durch Bluttransfusionen übertragen werden. Das Risiko einer Übertragung ist allerdings sehr gering, da alle Blutkonserven vor Gabe eingehend untersucht werden.

Ich bin darüber aufgeklärt worden, dass ich selbstverständlich die Möglichkeit habe, die Einwilligung zu verweigern und eine andere bereits bewährte Therapie durchführen zu lassen. Außerdem kann ich die Einwilligung jederzeit formlos widerrufen. Daraus entsteht für Patient und Sorgeberechtigten kein Nachteil.

Ich fühle mich genügend informiert. Im Fall einer Randomisation bin ich mit der Randomisation

O einverstanden.

O nicht einverstanden. Ich wünsche dann die Standardtherapie, die die Gabe von

Doxorubicin vorsieht.

Für weitergehende Fragen steht mir folgender Arzt in der Klinik zur Verfügung:

.....Telefon: .....

Eine Kopie dieser Einverständniserklärung wurde mir ausgehändigt.

Ich habe zum jetzigen Zeitpunkt keine weiteren Fragen.

....., den .....

.....

(Sorgeberechtigte, (Datum)

Patient selbst ab 16 Jahren oder bei

vorhandener Einsichtsfähigkeit früher)

.....

(gesprächsführender Arzt) (Datum)

.....

(Zeuge)(Datum)

Consent for data Storage and transfer

Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH

Einwilligungserklärung zur Speicherung und Auswertung personenbezogener medizinischer Daten

(zum Verbleib in der Krankenakte)

In dem Bemühen, die Behandlungsmethoden ständig zu verbessern, hat sich unsere Klinik mit anderen zusammengeschlossen, um möglichst viele und genaue medizinische Befunde aus den einzelnen Krankheitsverläufen zu dokumentieren, zu speichern und auszuwerten. Eine solche Dokumentation ist ein wichtiges Hilfsmittel einer zeitgemäßen Behandlung. Ziel ist es, die erkannten Verbesserungen in der Behandlung der Krankheit möglichst schnell vielen Kindern zugute kommen zu lassen. Ein wichtiger Bestandteil der Dokumentation kindlicher Krebserkrankungen ist das Deutsche Kinderkrebsregister am Institut für Medizinische Statistik und Dokumentation der Universität Mainz. Mit diesem arbeiten die behandelnden Kliniken eng zusammen. Darum ist es notwendig, dass personenbezogene und medizinische Daten auch an das Kinderkrebsregister übermittelt und dort elektronisch verarbeitet werden.

Um den Verlauf der Erkrankung erfassen zu können, ist es notwendig, die medizinischen Daten in personenbezogener Form zu speichern. Die Auswertungen erfolgen unter voller Wahrung der ärztlichen Schweigepflicht und des Datenschutzes. Nach den gesetzlichen Bestimmungen ist es erforderlich, dass zur Übermittlung und Speicherung personenbezogener Daten eine schriftliche Einwilligung gegeben wird. Ihr Einverständnis ist freiwillig. Selbstverständlich entstehen Ihnen für den Fall, dass Sie ihre Mitwirkung versagen, keinerlei Nachteile. Sie können Ihre Einwilligung jederzeit ohne Angaben von Gründen widerrufen. Bitte geben Sie uns durch Ihre Unterschrift im folgenden Ihre Einwilligung. Die Daten werden an folgende Zentren übermittelt:

Studienleitung:

Prof. Dr. Norbert Graf
Universitätsklinik für Kinder- und Jugendmedizin
Klinik für Pädiatrische Onkologie und Hämatologie
66421 Homburg / Saar

Deutsches Kinderkrebsregister
Langenbeckstraße 1
55101 Mainz

Im Rahmen notwendiger spezieller Maßnahmen werden Daten auch weitergegeben an:

Referenzradiologe SIOP 2001/GPOH
Prof. Dr. J. Tröger
Universitätskinderklinik Heidelberg
Abteilung Pädiatrische Radiologie
Im Neuenheimer Feld 150
69120 Heidelberg

Referenzstrahlentherapie SIOP 2001/GPOH
Prof. Dr. Ch. Rübe
Universitätsklinik für Strahlentherapie

Gebäude 49
66421 Homburg

Spätfolgenstudie LESS der GPOH
Prof. Dr. Beck
Abt. f. Päd. Hämatologie u. Onkologie
Universitätsklinik f. Kinder- u. Jugendl.
Loschgestr. 15
91054 Erlangen

Spätfolgen Strahlentherapie
Prof. Dr. N. Willich
Klinik Radioonkologie
Universitätsklinik der WWU
Albert Schweitzer Str. 33
48129 Münster

Referenzpathologie SIOP 2001/GPOH
Prof. Dr. I. Leuschner
Institut für Pathologie, Universität Kiel
Abteilung Paidopathologie
Michaelisstraße 11
24105 Kiel

Molekulargenetik des Nephroblastoms
Prof. Dr. M. Gessler
Physiologische Chemie I
Universität Würzburg
Am Hubland
97074 Würzburg

Im Rahmen der länderübergreifenden Zusammenarbeit der Behandlung kindlicher Tumoren werden die anonymisierten Daten (die Namen werden nicht übermittelt) an die Europäische Studienleitung der Nephroblastomstudie übermittelt:

Dr. Jan de Kraker
SIOP Nephroblastoma Trial & Study Office
Emma-Kinderziekenhuis / Het Kinder Academisch Medisch Centrum
Meibergdreef 9, room A3-273, P.O. Box 22660
NL-1105 AZ Amsterdam

Ebenso werden im Rahmen des Projektes **ACGT** (advanced clinicogenomic trials, gefördert von der Europäischen Union) anonymisierte Daten weitergegeben an:

Dr. Manolis Tsiknakis
FORTH
Vassilika Vouton, P.O. Box 1385
71110 Heraklion, Kreta
Griechenland

Prof. Dr. Meese
Institut für Humangenetik UdS
Gebäude 60
66421 Homburg

Alle Personen, die Einblick in die gespeicherten Daten haben, sind zur Wahrung des Datengeheimnisses verpflichtet. Die Auswertungen erfolgen unter voller Wahrung der ärztlichen Schweigepflicht und des Datenschutzes. Mein Einverständnis zu der Datenverarbeitung ist freiwillig. Für den Fall, dass ich meine Mitwirkung versage, entsteht mir hieraus kein Nachteil. Ich kann mein Einverständnis jederzeit widerrufen.

Ich erteile hiermit die Zustimmung zu der oben beschriebenen Datenübermittlung, Speicherung und Auswertung personenbezogener Daten. Ich habe diesbezüglich keine weiteren Fragen.

.....

Vor- und Nachname des Patienten

....., den .....

....

Unterschrift der Sorgeberechtigten Unterschrift des Patienten*

*zwingend ab 16 Jahren oder vorhandener Einsichtsfähigkeit jüngerer Patienten

....

gesprächsführender Arzt Zeuge

Consent for Asservation of biological material

Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH

Einwilligungserklärung zur Asservierung von Tumorgewebe

(zum Verbleib in der Krankenakte)

Ich bin damit einverstanden, dass Tumorgewebe meines Kindes zur Erforschung des Nephroblastoms in ihren molekularen, genetischen, immunologischen und anderen, mit der Krankheit direkt verbundenen Merkmalen untersucht und gegebenenfalls für die Entwicklung neuer Behandlungsverfahren eingesetzt wird. Die Entnahme des Tumorgewebes erfolgt schmerzlos im Rahmen der für mein Kind notwendigen operativen Tumorentfernung bzw. während der zur Diagnosestellung erforderlichen Probenentnahme aus dem Tumor. Falls bei der Tumorentfernung aus medizinisch chirurgischen Notwendigkeiten gesundes Gewebe mitentfernt werden muss, darf dieses als Vergleichsgewebe für die Tumoreigenschaften eingesetzt werden. Eine medizinisch nicht notwendige Erweiterung des operativen Eingriffes erfolgt dazu nicht. Zugestimmt wird der Entnahme einer Blutprobe während der Narkose (je nach Alter 2 – 10 ml) als Vergleichsmaterial für die Eigenschaften des Tumors. Tumor, Vergleichsgewebe und Vergleichsblut werden zentral in einer Tumorbank der GPOH (Gesellschaft für Pädiatrische Onkologie und Hämatologie) gelagert und kostenfrei und anonymisiert Wissenschaftlern, die in universitären Einrichtungen oder in Krankenhäusern tätig sind und in GPOH-Studien kooperativ eingebunden sind, für die obengenannten krankheitsbezogenen Untersuchungen zur Verfügung gestellt. Für die Nephroblastomstudie erfolgt die Lagerung in der Physiologische Chemie I (Direktor: Prof. Dr. M. Gessler) an der Universität Würzburg.

Ich bin damit einverstanden, dass bei meinem Kind zum Zeitpunkt der Diagnose, vor der Tumoroperation, nach der Tumoroperation und am Ende der Behandlung Blut entnommen wird, um diese auf Abwehrreaktionen gegen Antigene (bestimmte Eiweise) auf dem Tumor zu untersuchen.

Auf diese Weise sollen die Diagnosestellung sicherer gemacht werden, das biologische Verständnis der Erkrankung verbessert und neue therapeutische Ansätze gefunden werden.

.....

Vor- und Nachname des Patienten

....., den .....

....

Unterschrift der Sorgeberechtigten Unterschrift des Patienten*

*zwingend ab 16 Jahren oder vorhandener

Einsichtsfähigkeit jüngerer Patienten

....

gesprächsführender Arzt Zeuge

Statement of Participation to SIOP 2001/GPOH

Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH

Name des an der Klinik für die Nephroblastomstudie verantwortlichen Arztes :

.....

Klinik: .....

- **Patienten aller Stadien und jeden Alters mit einem Nephroblastom und alle Kinder mit einem Nierentumor werden in die Studie eingebracht.**
- **Die Protokollrichtlinien werden eingehalten unter Berücksichtigung der ärztlichen Verantwortung im Einzelfall.**
- **Die angeforderten Informationen, Präparate und Frischmaterialien (molekularbiologische Untersuchungen) werden von jedem Patienten zur Verfügung gestellt.**

Unterschrift

Kinderonkologe Name .....

Adresse .....

Tel.: ...../..... FAX: ...../.....

Email: @

Operateur Name .....

Adresse .....

Tel.: ...../..... FAX: ...../.....

Email: @

RadiotherapeutName .....

Adresse .....

Tel.: ...../..... FAX: ...../.....

Email: @

Pathologe Name .....

Adresse .....

Tel.: ...../..... FAX: ...../.....

Email: @

Bitte zurück an: SIOP 2001 / GPOH Nephroblastom Studie, Prof. Dr. N. Graf

- **Forms for Transmission**

Reference radiology

Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH

Absender (Stempel) .....200...
(Datum)

Bildgebende Diagnostik für die referenzradiologische Beurteilung

Patient..... geb.: .....

SIOP Nr. : _____PID : _____

Unsere Diagnose: .....

Sehr geehrter Herr Kollege,

bei o.g. Patient/in besteht der dringende V.a. ein Nephroblastom. Beiliegend übersenden wir Ihnen die bildgebende Diagnostik und unseren Befundbericht und bitten Sie als Referenzradiologe der Studie um die Mitbeurteilung.

Mit freundlichen Grüßen

Versand an: Prof. Dr. J. Tröger
Abt. für Pädiatrische Radiologie
Im Neuenheimer Feld 150
69120 Heidelberg

→ 2. Seite, Anhang mit notwendigen Informationen zur und für die Referenzradiologie

Anhang zum Formular Referenzradiologie

Die Referenzradiologie bittet um Zusendung der Schnittbildgebung des Abdomens **und** der Sonographie incl. schriftlicher Befunde (zumindest Kurzbefund der Sonographie), sowie um eine kurze Beantwortung des folgenden Fragebogens.

Zur wissenschaftlichen Auswertung bitten wir ferner um die Zusendung der **schriftlichen Befunde** der Thoraxbildgebung.

Welche Bildgebung wurde durchgeführt?
Wird sie vollständig oder als Auswahl zugesandt?

Durchgeführte Bildgebungdavon zur Referenzbeurteilung gesandt
O Ultraschall Abdomen**O** vollst.**O** Auswahl
O MRT Abdomen **O** vollst.**O** Auswahl
O CT Abdomen**O** vollst.**O** Auswahl
O Röntgen Thorax**O** vollst.**O** Auswahl**O** nein
O CT Thorax**O** vollst.**O** Auswahl**O** nein
O MIBG Szintigraphie**O** vollst.**O** Auswahl**O** nein

Anamnese und klinische Symptomatik

O Fieber **O** Harnwegsinfekt **O** Zufallsbefund **O** Trauma
O tastbarer Tumor **O** Sonst :
.....

.....

Relevante Laborwerte

Katecholamine : **O** normal**O** erhöht**O** nicht untersucht
Leukozytose : **O** ja **O** nein **O** nicht untersucht
Leukozyturie :**O** ja **O** nein **O** nicht untersucht
Anämie :**O** ja **O** nein **O** nicht untersucht
O Sonst:
.....

.....

Fragestellung / Zeitpunkt :

O Diagnose**O** Tumorvolumen vor OP**O** Verlauf von Metastasen
O Verdacht auf Rezidiv **O** Sonst :

.....

Besonderheiten (z.B. Metastasen, Cavathrombus)

.....

.....

Wurde die Chemotherapie bereits begonnen? **O** ja, am __.__200_ **O** nein

Molecular Biology

Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH

Absender (Stempel) .....200...
(Datum)

Material zur molekularbiologischen Untersuchung bei Nephroblastomen von:

Patient..... geb.: .....
SIOP Nr. :_____PID : _____
Operationsdatum.....200.....
Verdachtsdiagnose.....

□ Erstuntersuchung □ rechts □ links
□ unilateral□bilateral □ Nephroblastomatose
□ Syndrom
□ Beckwith-Wiedemann □Hemihypertrophie □ Perlman □Simpson-Golabi-Behmel □Sotos Syndrom
□ Denys-Drash □ Aniridie □ WAGR □ urogenitale Missbildung □ sonst :
_____
□ Familarität
□ primäre Operation □ nach präoperativer Chemotherapie
□ Ersterkrankung □ Rezidiv
□ Tumormaterial□Metastase□Normalgewebe
□- 70 °□- 70 °□ - 70 °
□_____□_____□ _____Proben__Proben__Proben

Bemerkungen :

……
KlinikstempelNameUnterschrift

Versand an: Prof. Dr. M. Gessler, Physiologische Chemie I, Universität Würzburg, Am Hubland
97074 Würzburg, Telefon : 0931 888 4159, Telefax : 0931 888 4150

Reference Pathology

Behandlung des Nephroblastoms entsprechend der Therapiestudie SIOP 2001 / GPOH

Absender (Stempel) .....200...
(Datum)

Histologische Präparate für die referenzpathologische Beurteilung

Patient..... geb.: .....

Operationsdatum.....200.....

unsere E.- Nr.:.....

Diagnose.....

Sehr geehrter Herr Kollege,

o.g. Patient/in wird im Rahmen der Nephroblastomstudie SIOP 2001 / GPOH behandelt. Beiliegend übersenden wir Ihnen die histologischen Präparate und unseren Befundbericht und bitten Sie als Referenzpathologen der Studie um die Mitbeurteilung.

Mit freundlichen Grüßen

Versand an:
Prof. Dr. D. Harms / Dr. I. Leuschner
Institut für Pathologie, Universität Kiel
Abteilung Paidopathologie
Michaelisstraße 11
24105 Kiel

Troponin, BNP And Doxorubicin Cmax

BEGLEITSCHEIN BLUTENTNAHME

Troponin, Brain Natriuretic Peptid (BNP) und Doxorubicin Cmax

Nephroblastomstudie SIOP 2001/GPOH

Name, Vorname | Pat.-Nr. | Klinik | Identifikationszahl | Geb.-Datum

GPOH-PID

Gewicht: ___,___ kg Größe: ___ cm Körperoberfläche: ___,___ m²

Datum der Doxorubicingabe: ___

Wievielte Doxorubicingabe: O 1. O 2. O 3. O 4. O 5. O 6.

Dosis: _____ mg Infusionsdauer : _____ Stunden

Bisherige kumulative Dosis: _____ mg

	Vor DOX	Ende DOX	24 h	48 h	Tag 5	Tag 21
Blutentnahme *	☐ Nein ☐	☐ Nein ☐	☐ Nein ☐	☐ Nein ☐	☐ Nein ☐	☐ Nein ☐
Doxorubicin						
Troponin						
BNP						

* 5 – 10 ml EDTA Blut, Plasma sofort bei –70° tieffrieren und an die Studienleitung senden:

Versand an:
Prof. Dr. Norbert Graf
Uni.-klinik für Kinder- und Jugendmedizin
Pädiatrische Onkologie und Hämatologie
Gebäude 9
66421 Homburg / Saar

_____
Einsendende Klinik

_____ _____ _____
Stempel Klinik Name Datum

Antigen Scenario

BEGLEITSCHEIN PROBENENTNAHME

Screening auf Antikörper gegen Nephroblastom-spezifische Antigene

Untersuchungsmaterial senden an:
Prof. Dr. N. Graf, Universitätsklinikum des Saarlandes, Klinik für Pädiatrische Onkologie und Hämatologie, Gebäude 9, 66421 Homburg

Name, Vorname | Pat.-Nr. | Klinik | Geb.Datum

Identifikationszahl

GPOH-PID

Blutentnahme (bitte Datum eintragen !)

	bei Diagnose vor Therapie	vor Operation nach präop. CT	6 Wochen nach Operation	1 Jahr nach Diagnose	anderer Zeitpunkt
Datum					
	bei Rezidiv vor Therapie	**bei erneuter Remission**	**bei erneuter Progression**	**1 Jahr nach Rezidivdiagnose**	**anderer Zeitpunkt**
Datum					

Resultate

Datum Lagerungsbeginn: Untersuchungsdatum:

Lagerung: **O** Raumtemperatur **O** Kühlschrank **O** < 0 °C **O** -80 °C

Labornummer: _____

Resultate:	**Gefundene Nephroblastom-spezifische Antigene**
Serum	

Einsendende Klinik

_____ _____ _____

Stempel Klinik Name Datum

Appendix 1: Abbreviations and acronyms

Act-D	Actinomycine D
ADR	Adriamycine
AE	Adverse Event
AFIP	Armed Forces Institute of Pathology
ANC	Absolute neutrophile count
CCSK	clear cell sarcoma of the kidney
cDNA	Complementary DNA
CIOMS	Council For International Organizations Of Medical Science
CMN	congenital mesoblastic nephroma
COG	Childrens Oncology Group
CPDN	Cystic partially differentiated nephroblastoma
DOPA	Dopamin
DOX	Doxorubicine
e.coli	Bacterium Escherichia coli
EFS	Event free survival
EMEA	European Agency for the Evaluation of Medicinal Products
EudraCT	European clinical trial database
G-CSF	Granulocyte-Colony Stimulating Factor
GraBCas	tool for score-based prediction of Caspase- and Granzyme B
Gy	Gray
HVA	Homovanillic acid
IDMC	Internal displacement monitoring centre
IMP	Investigational medical products
IVP	Intravenous pyelography
KEGG	Kyoto Encyclopedia of Genes and Genomes
L IV	4^{th} lumbal vertebra
LOH	loss of heterozygosity
MV	Megavolt
NRL	National Reference Laboratory
NWTSG	National Wilms Tumor Study Group
OS	Overall survival
P.A.	Posterior – anterior X-ray examination
PNET	Primitive neuroectodermal tumors
RT	Radiation Therapy
SAE	Severe Adverse Event
SEREX	Serological analysis of autologous tumor antigens
SIOP	International Society of Paediatric Oncology
SMART	Simple Modular Architecture Research Tool
SSD	Skin surface distance
SUSARs	Suspected Unexpected Severe Adverse Reactions
TH XII	12^{th} thoracic vertebra
UKCCSG	United Kingdom Children's Cancer Study Group
UKW	United Kingdom Wilmstumor
US	Ultrasonic
VCR	Vincristine
VMA	Vanillylmandelic acid
VOD	Veno occlusive desease
WBC	White blood cell count (leucocytes)
WHO	World Health Organisation

Appendix 2: Nephroblastoma Case Report Forms (CRFs)

MALIGNANT DISEASES* IN CHILDHOOD - REGISTRY

Gesellschaft für Pädiatrische Onkologie und Hämatologie (GPOH)
German Childhood Cancer Registry (GCCR)
at the Institute for Medizinische Biometrie, Epidemiologie und Informatik (IMBEI)

Please send the Registry Form to : GCCR at IMBEI, 55101 Mainz, Germany
Telephone: +49 6131/17-3227 or –6808, Fax: +49 6131/17-4462

Address of the Hospital, Telephone:

Patients Identification:

Identification No.: ⌊_____⌋

Last name: ⌊_____⌋ **Gender:** ⌊_⌋ 1=m, 2=f

First name: ⌊_____⌋ **Birth date:** ⌊_____⌋

Address (at the time of diagnosis):
Street: _____

Birth place or country where born: _____

Postal Code:_____ **City:** _____ **GPOH-PID:** ⌊_____⌋⌊_____⌋

Diagnosis: _____ **Localisation:** _____

_____ _____

Stage: _____ **Grading:** _____ **Date of diagnosis:** ⌊_____⌋

Diagnosis done by :
- ☐ Symptoms (incl. imaging studies)
- ☐ specific diagnostics (e.g. biochem./immunol. tests)
- ☐ Cytology
- ☐ Histology
- ☐ Autopsy
- ☐ unknown

Side:
- ☐ right
- ☐ left
- ☐ bilateral
- ☐ midline
- ☐ systemic disease
- ☐ unknown

Participation in a study: ☐ No ☐ Yes: **Name of study:** _____
(GCCR will send the registry form to the study office)

Written informed consent for data transfer to GCCR
- ☐ is given by the patient (mandatory for patients older than 16 years)
- ☐ is given by the parents or a person having the care and custody of the child
- ☐ was refused
- ☐ will be given soon

The parents information Sheet was handed out:
- ☐ No
- ☐ Yes – only below the age of 15 years -

_____ _____ _____
Name of the responsible physician (stmmp) **Date** **Signature**

*incl. benigne CNS Tumors, LCH, SAA and Second Malignancies

F1 - **1/2**

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH

REGISTRY FORM

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry (GCCR) at IMBEI, 55101 Mainz
Tel.: + 49 6131/17-3227 Fax: + 49 6131/17-4462

Name, Identification No.: | Pat.-No.: (no. in the study:) | Clinic | Identification No.: (IMBEI)

Please recognise further processing of this form needs the written informed consent for transmission and storage of data !

Birth date

GPOH-PID

Participation in a study : ☐ No ☐ **SIOP 2001/GPOH** ☐ Other : _____

Treatment in the hospital:
☐ Primary treatment
☐ Further treatment (Primary therapy in a national hospital)
☐ Further treatment (Primary therapy in a foreign country)

Pre-treatment in another hospital: ☐ No ☐ Yes, where: _____

Further therapy in another clinic : ☐ No ☐ Yes, where: _____

Reason for diagnostics:
☐ malignancy related ☐ prenatal diagnostics
☐ preventive medical check-up (U1 - U9, J1) ☐ provision of syndroms
☐ other reasons for diagnostics_____

Prior malignant disease (including systemic disease) ☐ No ☐ Yes, which one:_____

Syndrome/hereditary disease / associated malformations:
☐ No
☐ Yes: ☐ Aniridia ☐ WAGR Syndrome ☐ urogenital malformations
☐ Drash-Syndrome ☐ Wiedemann-Beckwith-Syndrome (EMG)
☐ Hemi-hypertrophy ☐ Perlman-Syndrome ☐ familiar Wilmstumour
☐ Other: _____ ☐ coagulation disorder
☐ Hypertonia, max. RR : ___/___ mm Hg

Family history of diseases (Leukamia, Tumour,or any congenital syndroms):
☐ No
☐ Yes, parents who: _____
☐ Yes, siblings who: _____
☐ Yes, others who: _____

Number of siblings ___ Multiples: ☐ No ☐ Yes
Type ☐ Twin ☐ Triplet / other multiples
Genesis ☐ monozygotic ☐ bizygotic

Birth date of the parents: Mother: 19__ Father: 19__

General Condition at diagnosis:
☐ Normal activity, no impairment
☐ Minor impairment of activity, no further help needed
☐ Major impairment of activity (no regular visit in school or kindergarden)
☐ Bed-ridden, in need of care
☐ Need of intensive care, moribund

Date of diagnosis by imaging ____

Begining of the protocol treatment: ____ with: ☐ Chemotherapy ☐ Surgery (not biopsy)
☐ other therapy: _____

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F1 - **2/2**

Localisation of the primary tumour: ☐ right ☐ left ☐ bilateral ☐ extrarenal

Metastases at diagnosis:
☐ no
☐ Yes: ☐ Lung ☐ Findings only by thoracic CT ☐ Mediastinal
☐ Liver ☐ extra-abdominal nodes ☐ Abdominal
☐ Bone ☐ Soft-tissue ☐ Brain
☐ Others, where: _____

Metastases detected by : Chest X-ray ☐ CT Chest ☐ CT Abdomen ☐
1: yes, 2: no, 3: n.d. US ☐ MRI ☐ other ☐

Number of lung metastases : Chest X-ray ☐ CT Chest ☐
maximum diameter of the largest lung metastases : ☐ mm

Catecholamine in urine in normal range: ☐ no ☐ yes ☐ not done

Reference radiology done : ☐ no ☐ yes

Tumour in the right kidney:

Please plot the precise localisation

Size of tumour	V = a [cm] x b [cm] x c [cm] x 0.523 [ml]			
	Length (a)	Width (b)	Thickness (c)	Volume (V)
Ultrasound				
CT-Scan				
MRI				

Imaging is compatible with: ☐ Wilmstumour ☐ Wilmstumour + NBL ☐ Nephroblastomatosis [NBL]
Number of tumours: ☐ Single tumour ☐ multiple tumours
Structure of the tumour: ☐ homogeneous ☐ inhomogene ☐ cystic
Biopsy done : ☐ No ☐ Yes ☐ Fine needle ☐ Trucut: Gauche: ☐
☐ open biopsy at (date) ☐☐☐☐☐☐

Tumour in the left kidney:

Please plot the precise localisation

Size of tumour	V = a [cm] x b [cm] x c [cm] x 0.523 [ml]			
	Length (a)	Width (b)	Thickness (c)	Volume (V)
Ultrasound				
CT-Scan				
MRI				

Imaging is compatible with: ☐ Wilmstumour ☐ Wilmstumour + NBL ☐ Nephroblastomatosis [NBL]
Number of tumours: ☐ Single tumour ☐ multiple Tumours
Structure of tumour: ☐ homogeneous ☐ inhomogeneous ☐ cystic
Biopsy done: ☐ No ☐ Yes ☐ Fine needle ☐ Trucut: Gauche: ☐
☐ open Biopsy at (date) ☐☐☐☐☐☐

Patient is protocol patient: ☐ Yes
☐ no, but observation group: Comment:
☐ Pat. is not of right age: ☐ < 6 Mon. ☐ > 16 Jahre
☐ Pat. has primary surgery, Reason: ☐ Emergency surgery
☐ Insecure diagnosis
☐ Deviation of the protocol
☐ other reasons: _____
☐ Pretreatment of the tumour
☐ Treatment impossible , Reason(s): _____
☐ bilateral tumour
☐ other tumour of the kidney (no Wilmstumour):_____
☐ Follow-up impossible: _____

Comment(s): Patient died: ☐ no ☐ yes

Stamp: Date: Signature:

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F2a - 1/2

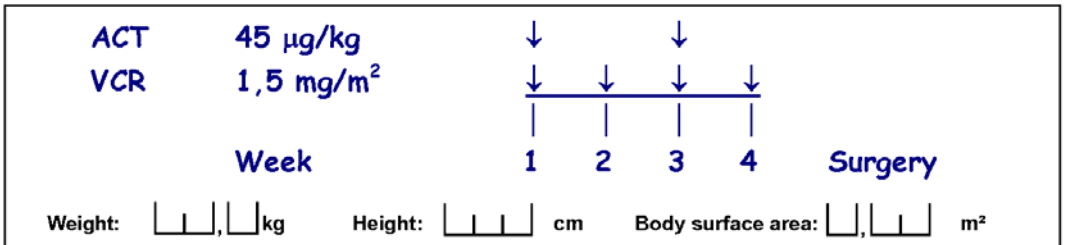
NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
PREOPERATIVE CHEMOTHERAPY - STAGE I, II, III, V AND IV-CT

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry (GCCR) at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID



Weight: ___,___ kg **Height:** ___ cm **Body surface area:** ___,___ m²

	Date	Weight [kg]	Administered dose ACT [µg]	Administered dose VCR [mg]	Dose-reduction	Reason?
Week 1					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 2					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 3					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 4					☐ No ☐ Yes: ___	☐ Toxicity ☐ other

Size of tumour after 4 weeks of preoperative chemotherapy (Evaluation of response)

Tumour in the right kidney: **Date of the evaluation of response :** ___

Size of tumour	**V = a [cm] x b [cm] x c [cm] x 0.523 [ml]** Length (**a**)	Width (**b**)	Thickness (**c**)	Volume (**V**)
Ultrasound				
CT-Scan				
MRI				

Structur of the tumour: ☐ homogeneous ☐ inhomogeneous ☐ cystic

Tumour in the left kidney: **Date of the evaluation of response :** ___

Size of tumour	**V = a [cm] x b [cm] x c [cm] x 0.523 [ml]** Length (**a**)	Width (**b**)	Thickness (**c**)	Volume (**V**)
Ultrasound				
CT-Scan				
MRI				

Structure of the tumour: ☐ homogeneous ☐ inhomogeneous ☐ cystic

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F2a - **2/2**

Toxicity or Severe Adverse Event (SAE): ☐ No ☐ Yes (please fill in form F8b !)

Venoocclusive Disease (VOD): ☐ No ☐ Yes (please fill in form F8b !)

In case of metastases in the lung found by CT-Scan alone and normal chest X-ray

Findings 4 Weeks after preoperative chemotherapy:

	Progress	Stable disease	> 50 % Regression	Complete remission
☐ Lung (Chest X-ray)	☐	☐		
☐ Lung (CT scan)	☐	☐	☐	☐

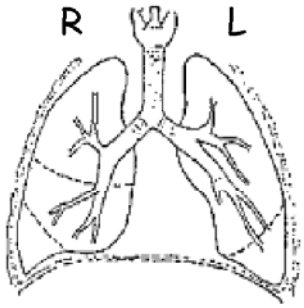
(Please plot the number of metastases and the precise localisation)

At diagnosis

☐ right ☐ left ☐ bilateral

Number: ⌊_⌋

Please plot the metastases

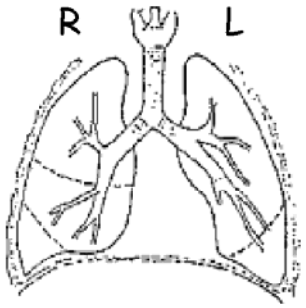


After preoperative chemotherapy

☐ right ☐ left ☐ bilateral

Number: ⌊_⌋

Please plot the metastases



Status after preoperative chemotherapy and surgery:

- ☐ No metastases after chemotherapy (CR)
- ☐ Complete resection of the metastases (please fill in form F3b)
- ☐ Incomplete resection of the metastases (please fill in form F3b) or multiple not-resectable metastases

Combined diagnosis of the changes found at the CT scan:

- ☐ Metastases
- ☐ Infected focus
- ☐ scar
- ☐ others:_____

Comment(s):

Stamp: Date: Siganture:

Version 3.0 / June 2006

F2b - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
PREOPERATIVE CHEMOTHERAPY - STAGE IV

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry (GCCR) at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no in the study: Clinic Identification No.: (IMBEI)

Birth date

PID

		Week 1	2	3	4	5	6	Surgery
ACT	45 µg/kg	↓		↓		↓		
VCR	1,5 mg/m²	↓	↓	↓	↓	↓	↓	
DOX	50 mg/m²	↓				↓		

Weight: ,kg Height: cm Body surface area: , m²

	Date	Weight [kg]	Administered dose ACT [µg]	VCR [mg]	DOX [mg]	Dose reduction	Reason ?
Week 1						☐ No ☐ Yes:	☐ Toxicity ☐ other
Week 2						☐ No ☐ Yes:	☐ Toxicity ☐ other
Week 3						☐ No ☐ Yes:	☐ Toxicity ☐ other
Week 4						☐ No ☐ Yes:	☐ Toxicity ☐ other
Week 5						☐ No ☐ Yes:	☐ Toxicity ☐ other
Week 6						☐ No ☐ Yes:	☐ Toxicity ☐ other

Toxicity or Severe Adverse Events : ☐ No ☐ Yes (Please fill in form **F8b** !)

Venoocclusive disease (VOD): ☐ No ☐ Yes (Please fill in form **F8b** !)

For the documentation of cardiotoxicity please always use form F8a

Version 3.0 / June 2006

F2b - **2/2**

Tumour size after 6 weeks of preoperative chemotherapy (Evaluation of Response)

Tumour in the right kidney: **Date of evaluation of response :** ☐☐☐☐☐☐

Size of tumour	Length (**a**)	Width (**b**)	Thickness (**c**)	Volume (**V**)
		V = a [cm] **x b** [cm] **x c** [cm] **x 0.523** [ml]		
Ultrasound				
CT-Scan				
MRI				

Strucure of the tumour: ☐ homogeneous ☐ inhomogeneous ☐ cystic

Tumour in the left kidney: **Date of evaluation of response :** ☐☐☐☐☐☐

Size of tumour	Length (**a**)	Width (**b**)	Thickness (**c**)	Volume (**V**)
		V = a [cm] **x b** [cm] **x c** [cm] **x 0.523** [ml]		
Ultrasound				
CT-Scan				
MRI				

Structure of the tumour: ☐ homogeneous ☐ inhomogeneous ☐ cystic

Metastases after 6 Weeks of preoperative chemotherapy:

	Progress	**Unchanged**	**> 50 % Regression**	**Complete Remission**
☐ Lung (X-ray chest)	☐	☐	☐	☐
☐ Lung (CT-Scan)	☐	☐	☐	☐
☐ Mediastinal	☐	☐	☐	☐
☐ Liver	☐	☐	☐	☐
☐ Abdominal	☐	☐	☐	☐
☐ Bone	☐	☐	☐	☐
☐ Brain	☐	☐	☐	☐
☐ Others: _____	☐	☐	☐	☐

Metastases of the lung: (Please plot number and localisation)

At diagnosis

☐ right ☐ left ☐ bilateral

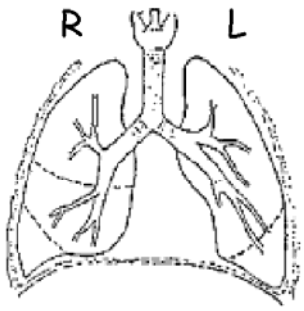
Number: ☐☐

After preoperative chemotherapy:

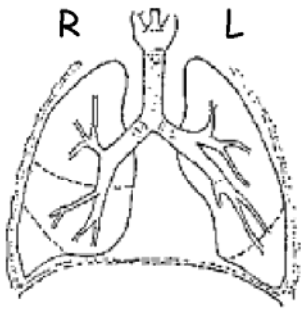
☐ right ☐ left ☐ bilateral

Number: ☐☐

Please plot the metastases



Please plot the metastases



Stage after preoperative chemotherapy and surgery:

☐ No metastases after chemotherapy (CR)
☐ Complete resection of the metastases (please fill in form F3b)
☐ Incomplete resection of the metastases (please fill in form F3b) or multiple not resectable metastases

Comment(s):

Stamp: Date: Signature:

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F3a - 1/4

- NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
OPERATIVE FINDINGS - PRIMARY TUMOUR

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry (GCCR) at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/ Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth Date

GPOH-PID

Date of surgery: **Surgeon:**

Surgeon : ☐ Pediatric surgeon ☐ Urologist ☐ Generel surgeon **Hospital:**

Postal code, city:

Number of patients with Nephroblastoma treated during the last two years :

in local hospital:	____ **or:**	☐ 1 - 5	☐ 5 -10	☐ more than 10
by the same surgeon:	____ **or:**	☐ 1 - 5	☐ 5 -10	☐ more than 10

Preoperative Treatment: ☐ initial surgery ☐ preoperative chemotherapy

Localisation of the primary tumour: ☐ right ☐ left ☐ bilateral * ☐ extra-renal
* please use a seperate form for each side

Intracaval extension of the tumour at diagnosis: ☐ No ☐ Yes ☐ infrahepatic ☐ suprahepatic ☐ retrohepatic ☐ intracardial

Intracavale extension of the tumour after preoperative CT: ☐ No ☐ Yes ☐ infrahepatic ☐ suprahepatic ☐ retrohepatic ☐ intracardial

Surgery with help of visceral surgeon: ☐ No ☐Yes

Approach: ☐ Midline ☐ Transverse / Chevron ☐ thoracoabdominal ☐ Longitudinal cut ☐ others: _____

Excision of suspicous area: ☐ Biopsy ☐ Tumourresection

	complete nephrektomy	**partial nephrektomy***
complete	☐	☐
incomplete	☐	☐
impossible	☐	

*** in case of partial nephrektomy:** ☐ partial nephrectomy ☐ "wedge resection" ☐ Enucleation

Stage defined by the surgeon: ☐ Stage I ☐ Stage II ☐ Stage III

Nodes affected macroscopically: ☐ No ☐ Yes

Version 3.0 / June 2006

F3a - **2/4**

Operative findings

Peritoneum

Appearance: ☐ normal ☐ suspicous adhesions ☐ Tumour adhesions ☐ Implant
Biopsy done: ☐ No Reason(s) : _____
Excision of suspected areas : ☐ complete ☐ incomplete ☐ simple biopsy only

Renal vein

Appearance: ☐ normal ☐ suspicious ☐ wall infiltrated ☐ Thrombosis
Biopsy done: ☐ No Reason: _____
Excision of suspected areas : ☐ complete ☐ incomplete ☐ simple biopsy only
Thrombectomy: ☐ complete ☐ incomplete ☐ not done

Vena cava

Appearance: ☐ normal ☐ suspicious ☐ wall infiltrated ☐ Thrombosis
Biopsy done : ☐ No Reason: _____
Excision of suspected areas: ☐ complete ☐ incomplete ☐ simple biopsy only
Thrombectomy: ☐ complete ☐ incomplete ☐ no
Cardio-pulmonary bypass: ☐ No ☐ Yes
Vena Cava Prothesis: ☐ No ☐ Yes

Tumour capsule

Appearence: ☐ normal ☐ suspicious ☐ apperently penetrated
Rupture: ☐ Yes : Time ☐ preoperative ☐ intraoperative
Type: ☐ major rupture ☐ simple fissure ☐ in dispute
☐ surgical biopsy

Lymph node

		regional	**extra-regional**
Appearence:	normal	☐	☐
	suspicious	☐	☐
	apperently penetrated	☐	☐
		regional	**extra-regional**
Excision of lymph nodes:	complete	☐	☐
	incomplete	☐	☐
	simple biopsy only	☐	☐
	no	☐	☐

if no, reason: _____
Rupture: ☐ No ☐ Yes
Number of lymph nodes resected: _____
Unusual intraabdominal finding: ☐ No ☐ Yes
If yes, which one: _____

Version 3.0 / June 2006

F3a - 3/4

Any other structure or organ suspicious or invaded outside the resected kidney ?

1. Column: **Organ** — suspicious/infiltrated: [1] Metastases/Implant: [2] normal: [3] not seen: [4]

2. Column: **Resection of suspicous areas:** — complete : [1] incomplete : [2] Biopsy only: [3] not sampled : [4]

Adrenal	[ ]	[ ]	Spleen	[ ]	[ ]
Peri-renal Fat	[ ]	[ ]	Pancreas	[ ]	[ ]
Ureter	[ ]	[ ]	Colon-mesocolon	[ ]	[ ]
Liver	[ ]	[ ]	contralateral kidney	[ ]	[ ]
Psoas	[ ]	[ ]	other:	[ ]	[ ]
Peritoneum	[ ]	[ ]	which one : _____		

Complication

Complication (intraoperative):	☐ No	☐ Yes	☐ unknown, no data
Tumour rupture: minor	☐	☐	☐
major	☐	☐	☐
Bleeding (> 50 ml/kg)	☐	☐	☐
Hypotension	☐	☐	☐
Cardiac arrest	☐	☐	☐
Vascular injury	☐	☐	☐
Bowel infarction	☐	☐	☐
Bowel injury	☐	☐	☐
Spleenic injury	☐	☐	☐
Liver injury	☐	☐	☐
Others	☐	☐	☐

Which one: _____

All surgical related complications and further surgeries must be reported up to one year after surgery →F3_K

Complication (postoperative):	☐ No	☐ Yes	☐ unknown, no data
Postoperative Bleeding	☐	☐	☐
V. cava obstruction	☐	☐	☐
Ileus due to adhesions	☐	☐	☐
Ileus due to intussusception	☐	☐	☐
Wound infection	☐	☐	☐
Wound dehiscence	☐	☐	☐
Incisional hernia	☐	☐	☐
Diaphragmatic hernia	☐	☐	
Others	☐	☐	☐

In case of postoperative complication:
Date of complication: |_|_|_|_|_|_|

Which one: _____

(Resection of other visceral organs at time of nephrectomy (due to injury): ☐ No ☐ Yes

If yes, which one: ☐ Liver ☐ Pancreas ☐ Diaphragm ☐ Spleen ☐ Colon ☐ others: _____

Resection is necessary because of: ☐ Injury ☐ Radicality of the tumour resection

Treatment of complication:

Medical only:	☐ No	☐ Yes	☐ unknown
Surgical at time of nephrectomy:	☐ No	☐ Yes	☐ unknown
Surgical (Reoperation):	☐ No	☐ Yes	☐ unknown

Date of reoperation: |_|_|_|_|_|_|

Version 3.0 / June 2006

F3a - 4/4

Result of surgery:

Death: ☐ No ☐ Yes ☐ unknown

If yes, concrete reason: _____

Sequelae: ☐ No ☐ Yes ☐ unknown

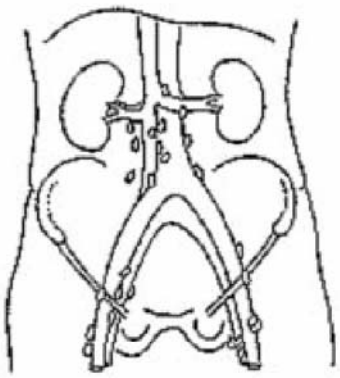
If yes, which one: _____

Delay in chemotherapy/radiotherapy: ☐ No ☐ Yes ☐ unknown

If yes, number of days: ☐☐☐

Please plot the extension of the tumour and localisation of the resected lymph nodes !

Please sent a copy of the surgical report together with the resected material to the local Pathologist. The tumour material is only allowed to be opened after the Pathologist staged the tumour. The tumour material for molecular genetic examination has to be taken in cooperation with the local Pathologist. Sent the material according to the protocol to the Wilms tumour data bank.



Comment(s):

Stamp: Date: Siganture of surgeon:

Attention !

This form must be filled out by the attending Surgeon after the surgery directly.
The form and a copy of the surgical report should be sent to the study office.

In case of bilateral Nephroblastoma a seperate form should be filled in for each site.
A seperate form should be filled in (F3b) for each further complication or metastases surgery.

Complication caused by the surgery in the duration of one year after initial tumour surgery should be filled in a seperate form by the Oncolgist-in-charge of the patient.

Version 3.0 / June 2006

F3a_K - 1/1

- NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -

SURGERY FORM – COMPLICATIONS POSTOPERATIVE

Study office: Prof. Dr. N. Graf, University hsopital of the Saarland, 66421 Homburg/Saar, Germany
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz, Germany
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study | Clinic | Identification No.: (IMBEI)

Birth Date

GPOH-PID

Date of the postoperative complication(s) :

All surgical related complications and further surgeries must be reported up to one year after surgery F3_K

Complication (postoperative):	☐ No	☐ Yes	☐ unknown, no data
Postoperative Bleeding	☐	☐	☐
V. cava obstruction	☐	☐	☐
Ileus due to adhesion	☐	☐	☐
Ileus due to intussusception	☐	☐	☐
Wound infection	☐	☐	☐
Wound dehiscence	☐	☐	☐
Incisional hernia	☐	☐	☐
Diaphragm hernia	☐	☐	☐
Others	☐	☐	☐

Others: _____

Treatment of the complication(s):

Medical only : ☐ No ☐ Yes ☐ unknown

Surgical (Reoperation): ☐ No ☐ Yes ☐ unknown

Date of the Reoperation:

Resection of other visceral organs: ☐ No ☐ Yes

If yes, which one: ☐ Liver ☐ Pancreas ☐ Diaphragm ☐ Spleen ☐ Colon ☐ others: _____

Result of the surgery:

Death: ☐ No ☐ Yes ☐ unknown

If yes, concrete reason: _____

Sequelae: ☐ No ☐ Yes ☐ unknown

If yes, which one: _____

Delay in chemotherapy/radiotherapy: ☐ No ☐ Yes ☐ unknown

If yes, Number of days:

Comments:

Stamp: Date: Signature of the oncologist

Version 3.0 / June 2006

F3b - 1/1

NEPHROBLASTOM - CLICICAL TRIAL & STUDY SIOP 2001/GPOH -
SURGERY FORM - METASTASES

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

Date of the surgery:

Surgeon: ☐ Pediatric surgeon ☐ Thoracic surgeon ☐ General surgeon ☐ Urologist

Surgeon: _____

Hospital: _____

Postal code, city: _____

Indication: curative ☐ palliative ☐

Preoperative Treatment: Chemotherapy Yes ☐ No ☐; Radiotherapy Yes ☐ No ☐; Metastectomy Yes ☐ No ☐

Localisation of Metastases: Lung ☐ Bone ☐ CNS ☐ Liver ☐ Soft tissue ☐ Others ☐

Precise localisation: _____

Appearence of the Metastases in relation to the Primary tumour: synchrone☐ metachrone☐

Site: right ☐ left ☐

Metastectomy performed in: Lung ☐ Bone ☐ CNS ☐ Liver ☐ Soft tissue ☐ Others ☐

Adhesions: No ☐ Yes ☐

Excision: complete ☐ Number ; incomplete ☐ Number ; Biopsy only ☐

Precise excision details for lung and liver: Wedge Resection ☐ Segmentectomy ☐ Lobectomy ☐ Pneumonectomy ☐ Transplantation ☐

Further treatmwnt: none ☐ Re-OP ☐ Chemotherapy ☐ Radiotherapy ☐ Stem cell transplantation ☐

This form must be filled out by the attending surgeon directly after the surgery. A copy of the form and the surgical report should be sent to the study office . A seperate form should be filled in (F3b) for each Metastectomy

Comment(s):

Stamp: Date: Signature

Version 3.0 / June 2006

F4 - 1/4

- NEPHROBLASTOM - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
PATHOLOGY FORM

Studienleitung: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: 06841 16-28397 Fax: 06841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

Date of reporting: local Pathologist:

Pathology ID No.: Hospital:

Postal code,City:

For each site of the tumour a seperate form should be filled in and sent to the study office !

Information of the surgery received by pathologist Yes ☐ No ☐

Preoperative chemotherapy ☐ Primary nephrectomy ☐

Tumour side right ☐ left ☐ bilateral ☐

Tumour material
- unilateral: complete ☐ — partial nephrectomy ☐
- bilateral: left ☐ — complete nephrectomy ☐
- bilateral: left ☐ — partial nephrectomy ☐
- bilateral: right ☐ — complete nephrectomy ☐
- bilateral: right ☐ — partial nephrectomy ☐

Specimen Weight: g

Largest tumour diameter-multifocal, indicate the diameter of the largest single tumour , cm

Specismen received intact and unopened from operating theatre? Yes ☐ No ☐ Uncertain ☐

Renal capsule grossly intact ? Yes ☐ No ☐ Uncertain ☐

Surface inked ? Yes ☐ No ☐ Uncertain ☐

Tumour multifocal ? Yes ☐ No ☐ Uncertain ☐

Comment(s):

Resection margin involved by tumour ?
- macroscopically Yes ☐ Necrosis ☐ No ☐ Uncertain ☐
- microscopically Yes ☐ Necrosis ☐ No ☐ Uncertain ☐

if yes , comment(s):

Renal vein thrombus ?
- macroscopically Yes ☐ No ☐ Uncertain ☐
- microscopically Yes ☐ No ☐ Uncertain ☐

Version 3.0 / June 2006

F4 - 2/4

Percentage of necrosis/regressive changes on gross examination:

< 65 % ☐ state: ☐☐ % 65 - 99 % ☐ state: ☐☐ % 100 % ☐

Percentage of necrosis/regressive changes on histological examination:

< 65 % ☐ state: ☐☐ % 65 - 99 % ☐ state: ☐☐ % 100 % ☐

Nephrogenic rests ? yes ☐ no ☐ uncertain ☐

If anaplastic Nephroblastoma, please subclassify focal ☐ diffuse ☐ uncertain ☐

Local histological diagnosis (Revised SIOP Classification of kidney tumours in childhood, 2001):

		Preop. CT	prim. OP
LOW RISK TUMOUR	☐		
Mesoblastic Nephroma		☐	☐
Cystic Partially Differentiated Nephroblastoma (CPDN)		☐	☐
Completely Necrotic Nephroblastoma		☐	
INTERMEDIATE RISK TUMOUR	☐		
Nephroblastoma - epithelial type		☐	☐
Nephroblastoma - stromal type		☐	☐
Nephroblastoma - mixed type		☐	☐
Nephroblastoma - regressive type		☐	
Nephroblastoma - focal Anaplasia		☐	☐
Nephroblastoma - blastemal Type			☐
HIGH RISK TUMOUR	☐		
Nephroblastoma - Blastemal type		☐	
Nephroblastoma - diffuse anaplasia		☐	☐
Clear Cell Sarkoma of the kidney (CCSK)		☐	☐
Rhabdoid Tumour of the kidney		☐	☐
NOT CLASSIFIABLE	☐		
NEPHROBLASTOMATOSIS	☐		
OTHER MALIGNANT TUMOUR	☐		
Primitive Neuroectodermal Tumour (PNET)		☐	☐
Cell Carcinoma of the kidney		☐	☐
Others: _____		☐	☐
OTHER BENIGN TUMOURS OR LESIONS	☐		
Cystic Nephroma		☐	☐
Adenoma		☐	☐
Diagnosis: _____		☐	☐

Lymph nodes (hilar, peri-aortic or other abdominal sites) ?

Positive for tumour No ☐ Uncertain ☐ Not examined ☐

Yes ☐ Vital ☐ Necrosis ☐

if yes, LN-localisation and comment(s): _____

Number of lymphnodes resected : _____ **with viable or non-viable metastases :** _____

Locale SIOP Stage (check staging at page 4)

STAGE I ☐

STAGE II ☐ Reason(s): _____

STAGE III ☐ Reason(s): _____

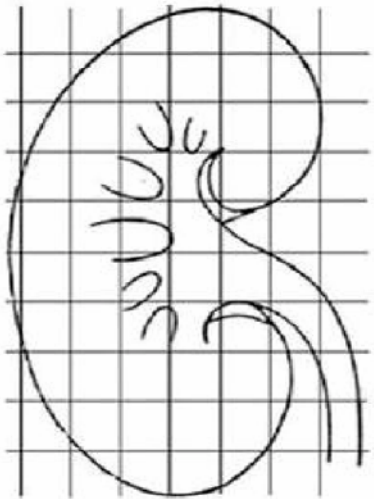
Version 3.0 / June 2006

F4 - 3/4

Material stored for biological studies ? No ☐ Yes ☐ uncertain ☐
sent ? No ☐ Yes ☐ uncertain ☐

Histological examination of the bordering tissue by "mirror blocks" ? No ☐ Yes ☐

Right kidney



Left kidney

Please draw or photograph the tumour and document the exact site by using numbers and letters for each section taken.

Comment(s):

Stamp: Date: Signature of the pathologist:

Attention !

This form should be filled in by the local Pathologist. The report of the histology is to be sent to the study office in copy. Please submit a full set of H&E slides and one paraffin block or the half of the specimen to the refernce pathologist to Kiel/ Germany

Version 3.0 / June 2006

F4 - 4/4

SIOP STAGING CRITERIA

Stage I

a) The tumour limited to kidney or surrounded with a fibrous pseudocapsule if outside of the normal contour of kidney, the renal capsule or pseudocapsule may be infiltrated with the tumour but it does not reach the outer surface, and it is completely resected (resection margins 'clear')
b) The tumour may be protruding ("bulging") into the pelvic system and dipping into the ureter (but it is not infiltrating their walls).
c) The vessels of the renal sinus are not involved
d) Intrarenal vessel involvement may be present

Fine needle aspiration or percutaneous core needle biopsy ('tru-cut') does not upstage the tumour but the size of the needle gauge should be mentioned to the pathologist.
The presence of necrotic tumour or chemotherapy-induced changes in the renal sinus and/or within the perirenal fat should not be regarded as a reason for upstaging a tumour providing it is completely excised and does not reach the resection margins.

Stage II

a) The tumour extends beyond kidney or penetrates through the renal capsule and/or fibrous pseudocapsule into peri-renal fat, but is completely resected
b) The tumour infiltrates the renal sinus and/or invades blood and lymphatic vessels outside the renal parenchyma but it is completely resected
c) Tumour infiltrates adjacent organs or vena cava but is completely resected

Stage III

a) Incomplete excision of the tumour which extends beyond resection margins (gross and/or microscopical tumour remains post-operatively)
b) Any abdominal lymph nodes are involved
c) Tumour rupture pre- or intra-operatively (irrespective of other criteria for staging)
d) The tumour has penetrated through the peritoneal surface
e) Tumour implants are found on the peritoneal surface
f) Tumour thrombi present at resection margins of vessels or ureter, transected or removed piecemeal by surgeon
g) The tumour has been surgically biopsied (wedge biopsy) prior to preoperative chemotherapy or surgery

The presence of necrotic tumour or chemotherapy-induced changes in a lymph node or at the resection margins is regarded as a proof of previous tumour with microscopic residue and therefore the tumour is assigned stage III (because of a possibility that some viable tumour is left behind in the adjacent lymph node or beyond resection margins.)

Stage IV

Haematogeneous metastases (lung, liver, bone, brain, etc) or lymph node metastases outside of the abdomino-pelvic region

Stage V

Bilateral renal tumours at diagnosis. Each side should be substaged according to the above classifications.

Version 3.0 / June 2006

Randomisation Form (F5)

Please fill in this form as soon as consent for randomisation is obtained and sent directly the form to the study office after the reference of the histological examination is available !

Egilibility criteria for randomisation	Yes	No
Age > 6 months and < 16 years	☐	☐
Unilateral tumour	☐	☐
Absence of metastases	☐	☐
Preoperative chemotherapy according to protocol	☐	☐
Stage II or III, epithilial, stromal subtype or other intermediate risk and < 500 ml tumourvolume	☐	☐
Normal findings in echocardiography	☐	☐
Post-operative treatment possible	☐	☐
Surveillance possible for 2 years or more	☐	☐
Informed consent of parents	☐	☐

Patient identification

Name (First five letters) |_|_|_|_|_|

Birth date |_|_|_|_|_|_|_|_|

Date of surgery |_|_|_|_|_|_|_|_|

Pat.-No. / I-No. in the study |_|_|_|_|

Centre identification

Name of physician .....

Treatment centre .....

City .....

Street .....

Phone .....

Fax .....

Email .....

Study office:

Prof. Dr. Norbert Graf
University hospital of the Saarland
Pediatric Oncology und Haematology
66421 Homburg / Saar, Germany
Phone: 06841/16 28047, 28399
Fax : 06841/16 28302
Email: norbert.graf@uniklinikum-saarland.de

Version 3.0: June 2006

Result of randomisation

Date of randomisation

Name of physician .....

Treatment centre .....

City .....

Street .....

Phone .....

Fax .....

Email .....

Patient identification

Name .....

Birth date |_|_|_|_|_|_|_|_|

Pat.-No./I-No. in the study |_|_|_|_|

Result of randomisation:

Postoperative chemotherapy for stage II or III and epithelial or stromal subtype or other intermediate risk tumours and tumour volume < 500 ml:

☐ **with Doxorubicin**

☐ **without Doxorubicin**

Date of randomisation : ___.___._____

Prof. Dr. Norbert Graf
Chairman of the trial and study

Version 3.0: June 2006

F6 - 1/3

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
POST-OPERATIVE RADIOTHERAPY

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

Radiotherapist`s Name: _____

Radiotherapy centre: _____

Did patient receive abdominal radiotherapy ? ☐ No ☐ Yes, if yes:

Radiotherapy started at: ____ **Radiotherapy ended at:** ____

Device/Apparatus: ☐ Cobalt 60 ☐ Linear accelerator (Photons): ☐ < 9 MeV ☐ ≥9 MeV

Others: _____

Fields of the abdominal RT:

1. Primary field: ☐ Tumour bed ☐ Tumour + para-aortic LN ☐ abdominal bath
2. Shielding: ☐ no, reason(s): _____
   ☐ Liver ☐ contralateral kidney ☐ Liver and kidney
3. Boost ☐ No
   ☐ Yes: Localisation: _____
   Shielding: ☐ No ☐ Yes

Dose:
Total midplane dose: ___,___ Gy Single dose: ___,___ Gy
Number of fractions : ____ Duration in days: ____
Interruption in days: ____ Reason(s): _____

_____

If shielding is used : administered dose at liver : ___,___ Gy
administered dose at opposite kidney: ___,___ Gy

If boost is used: administered dose of boost: ___,___ Gy
Number of fractions: ____ Duration in days: ____
Interruption in days: ____ Reason(s): _____

_____

Version 3.0 / June 2006

F6 - **2/3**

Toxicity

Nausea	☐ No	☐ mild	☐ moderate	☐ severe
Emesis	☐ No	☐ mild	☐ moderate	☐ severe
Hepatic toxicity	☐ No	☐ abnorme	☐ clinical	☐ yes, specify:

Laboratory value _____

Other kind of toxicity _____

Nadir Blood cell count

Hb	Lymphocytes	Neutrophiles	Platelets

Radiation to the lung

Did patient receive radiotherapy to the lung? ☐ No ☐ Yes, if yes:

Radiotherapy started at: |_|_|_|_|_| **Radiotherapy ended at:** |_|_|_|_|_|

Dose:

Total dose: |_|_|,|_| Gy Single dose: |_|_|,|_| Gy

Number of days patient received RT |_|_|_| Duration in days: |_|_|_|

Interruption in days: |_|_| Reason(s): _____

_____

Radiotherapy to other localisation

Did the patient receive radiotherapy to any other area ? ☐ No ☐ Yes, when:

Radiotherapy started at: |_|_|_|_|_| **Radiotherapy ended at:** |_|_|_|_|_|

Localisation : ☐ Bone: _____

☐ Brain: _____

☐ Other: _____

_____

Dose:

Administered total dose: |_|_|,|_| Gy Single dose: |_|_|,|_| Gy

Number of days patient received RT: |_|_|_| Duration in days: |_|_|_|

Interruption in days: |_|_| Reasons(s) _____

_____

Version 3.0 / June 2006

F6 - 3/3

Measurement of fields:

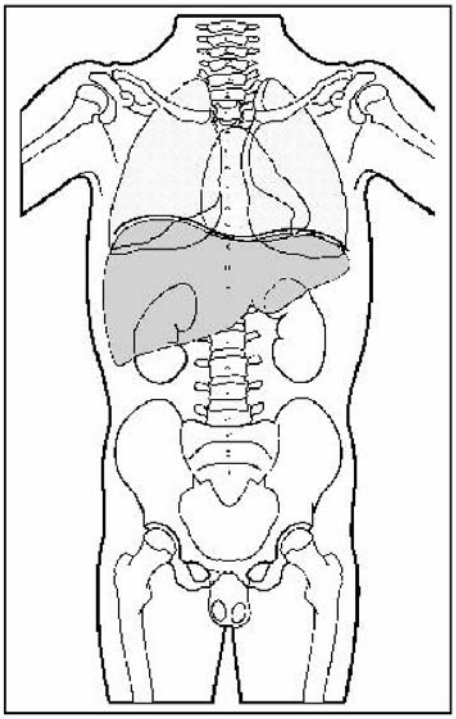
Please draw the shields and indicate shielding

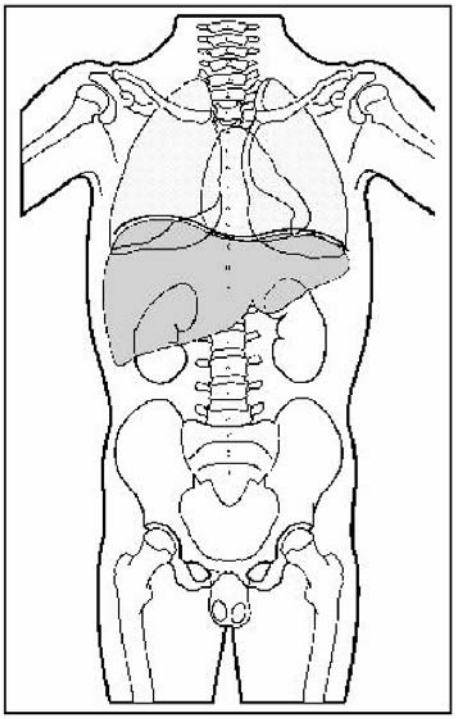
Primary field

Fields	Length	Width
Anterior		
Posterior		
Other		

Boost

Fields	Length	Width
Anterior		
Posterior		
Other		





Please enclose copy of the simulation films of target and boost volume !

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7a - 1/1

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -

POST-OPERATIVE CHEMOTHERAPY - AV-1

Studiy office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

ACT 45 µg/kg
VCR 1,5 mg/m²
Weeks 1 2 3 4

Stage I, epithelial-, stromal, other intermediate risk < 500 ml ☐ Yes ☐ No

Weight: ___,___ kg **Height:** ___ cm **Body surface area:** ___,___ m²

	Date	Weight [kg]	Administered dose ACT [µg]	Administered dose VCR [mg]	Dose-reduction	Reason ?
Week 1					☐ No ☐ Yes ___	☐ Toxiciy ☐ other
Week 2					☐ No ☐ Yes ___	☐ Toxicity ☐ other
Week 3					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 4					☐ No ☐ Yes ___	☐ Toxicity ☐ other

Toxicity or Severe Adverse Events : ☐ No ☐ Yes (please fill in form **F8b** !)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b** !)

At the end of treatment:
☐ complete remission (CR)
☐ partial remission (PR)
☐ no change (NC)
☐ Progression (PD)
☐ unable to determine

Postoperative complications: _____

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7b - 1/2

- NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
POSTOPERATIVE CHEMOTHERAPY - RANDOMISATION

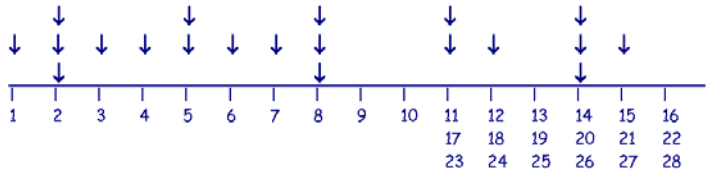
Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. (I-no. in the study:) Clinic Identification No.: (IMBEI)

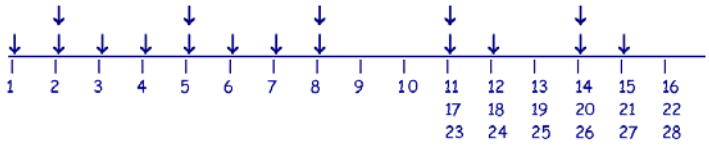
Birth date

GPOH-PID

ARM A



ARM B



Patient can be randomised: ☐ Yes ☐ No

Patient is randomised : ☐ Yes

☐ No reason(s): ☐ Refusal by patient/parents
☐ Refusal by clinic
☐ med. reasons: ☐ Toxicity ☐ Metastases
☐ logistical reasons
☐ others: _____

Randomisation arm : ☐ with Doxorubicin ☐ without Doxorubicin

Patient received therapy arm: ☐ with Doxorubicin ☐ without Doxorubicin
☐ others: _____

Toxicity or Severe Adverse Events: ☐ No ☐ Yes (please fill in form **F8b!**)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b!**)

If patient received RT, did the RT aggravate the SAE: ☐ No ☐ Yes

At the end of treatment:
☐ complete remission (CR)
☐ partial remission (PR)
☐ no change (NC)
☐ Progression (PD)
☐ unable to determine

Postoperative complication: _____

For the documentation of cardiotoxicity please always use the form F8a

Version 3.0 / June 2006

F7b - **2/2**

Weight: ___,___ kg **Height:** ___ cm **Body surface area:** ___,___ m²

	Date	Weight [kg]	Administered dose ACT [µg]	VCR [mg]	DOX [mg]	Dose-reduction	Reason ?
Week 1						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 2						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 3						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 4						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 5						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 6						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 7						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 8						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 11						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 12						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 14						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 15						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 17						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 18						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 20						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 21						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 23						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 24						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 26						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 27						☐ No ☐ Yes: ___	☐ Toxicity ☐ other

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7c - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -

POST-OPERATIVE CHEMOTHERAPY - AVD, AV-2

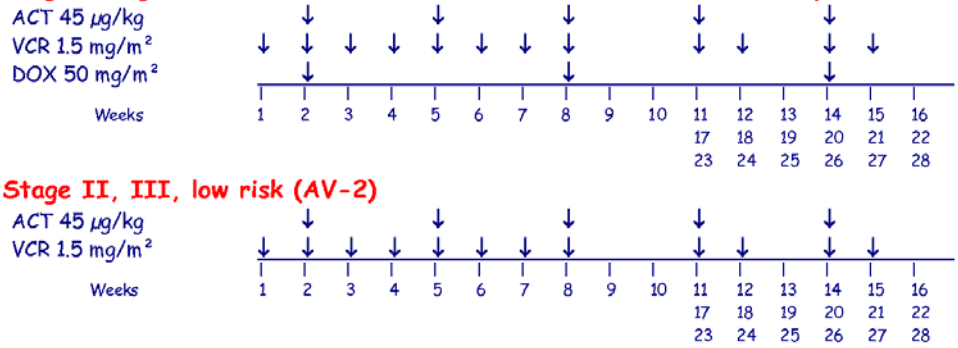
Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No. : Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth Date

GPOH-PID

Stage I, high, intermediate risk and ≥500 ml (without: stromal-, epithelial)



Stage II, III, low risk (AV-2)

local stage: ☐ I ☐ II ☐ III
Histology: ☐ low ☐ intermediate ☐ high risk ☐ epithelial ☐ stromal
Tumour volume: ☐ < 500 ml ☐ ≥ 500 ml

Toxicity or Severe Adverse Events: ☐No ☐ Yes (please fill in form **F8b** !)

Venoocclusive disease (VOD): ☐No ☐ Yes (please fill in form **F8b** !)

At the end of treatment:
☐ complete remission (CR)
☐ partial remission (PR)
☐ no change (NC)
☐ Progression (PD)
☐ unable to determine

Postoperative complications: _____

For the documentation of cardiotoxicity please always use the form F8a

Version 3.0 / June 2006

F7c - **2/2**

Weight: ☐☐,☐ **kg** **Height:** ☐☐☐ **cm** **Body surface area:** ☐,☐☐ **m²**

	Date	Weight [kg]	Administered dose ACT [µg]	VCR [mg]	DOX [mg]	Dose-reduction	Reason ?
Week 1						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 2						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 3						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 4						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 5						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 6						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 7						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 8						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 11						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 12						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 14						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week15						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 17						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 18						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 20						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 21						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 23						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 24						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 26						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 27						☐ No ☐ Yes: ___	☐ Toxicity ☐ other

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7d - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
POSTOPERATIVE CHEMOTHERAPY - VP16/CARBO/CYCLO/DOX

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-No. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

VP16	150 mg/m²	↓↓↓ ↓↓↓ ↓↓↓ ↓↓↓
CARBO	200 mg/m²	↓↓↓ ↓↓↓
CYCLO	450 mg/m²	↓↓↓ ↓↓↓ ↓↓↓
DOX	50 mg/m²	↓ ↓ ↓

RT

Weeks 1<----2-----3---> 4 5 6 7 8 9 10 11 12 13 14 15
16 17 18 19 20 21 22 23 24 25 26 27
28 29 30 31 32 33 34

Weight: ___,___ kg **Height:** ___ cm **Body surface area:** ___,___ m²

	Date	Weight [kg]	Administered dose VP16 [mg]	CARBO [mg]	CYCLO [mg]	DOX [mg]	Dose-reduction	Reason ?
We 1, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 4, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 7, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 10, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 13, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 16, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 19, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 22, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 25, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 28, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 31, d1 d2 d3							☐ No ☐ Yes:	☐ Toxicity ☐ other
We 34, d1 d2 d3							☐ No ☐ Yes:	☐ Toxicity ☐ other

Version 3.0 / June 2006

F7d - **2/2**

Toxicity or Severe Adverse Event : ☐ No ☐ Yes (please fill in form **F8b** !)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b** !)

If patient received RT, did the RT aggravate the SAE: ☐ No ☐ Yes

Was G-CSF administered to the patient: ☐ No ☐ Yes

At the end of treatment:

- ☐ complete remission (CR)
- ☐ partial remission (PR)
- ☐ no change (NC)
- ☐ Progression (PD)
- ☐ unable to determine

Postoperative complication: _____

For the documentation of cardiotoxicity please always use the form F8a

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7c4 - 1/1

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
POSTOPERATIVE CHEMOTHERAPY - AVD - STAGE IV

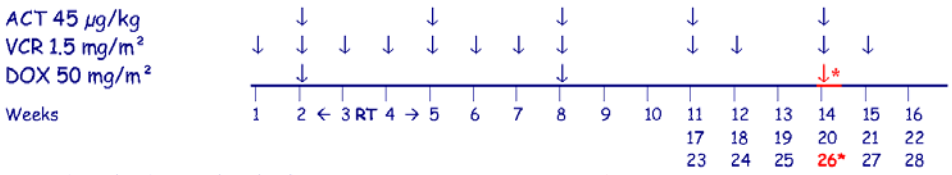
Study office: Prof. Dr. N. Graf,Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study : Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

Local stage I, II, III, CR of metastases post-operative
Low risk



RT: Abdominal radiation only at local stage III; no Doxorubicin in week 26*

local stage: ☐ I ☐ II ☐ III
Histology: ☐ low ☐ intermediate ☐ high risk ☐ epithelial ☐ stromal
Tumour volume: ☐ < 500 ml ☐ ≥ 500 ml

Toxicity or Severe Adverse Events : ☐ No ☐ Yes (please fill in form **F8b** !)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b** !)

If patient received RT, did RT aggravate the event: ☐ No ☐ Yes

At the end of treatment:
☐ complete remission (CR)
☐ partial remission (PR)
☐ no change (NC)
☐ Progression (PD)
☐ unable to determine

Postoperative complications: _____

For the documentation of cardiotoxicity please always use the form F8a

Version 3.0 / June 2006

F7c4 - 2/2

Weight: ☐☐,☐ kg Height: ☐☐☐ cm Body surface area: ☐,☐☐ m²

	Date	Weight [kg]	Administered dose ACT [µg]	VCR [mg]	DOX [mg]	Dose-reduction	Reason ?
Week 1						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 2						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 3						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 4						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 5						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 6						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 7						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 8						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 11						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 12						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 14						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 15						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 17						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 18						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 20						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 21						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 23						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 24						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 26						☐ No ☐ Yes: ____	☐ Toxicity ☐ other
Week 27						☐ No ☐ Yes: ____	☐ Toxicity ☐ other

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7d4 - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -

POST-OPERATIVE CHEMOTHERAPY - VP16/Carbo/Cyclo/DOX -STAGE IV

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.:(IMBEI)

Birth date

GPOH-PID

		Weeks
VP16	150 mg/m²	↓↓↓ ↓↓↓ ↓↓↓ ↓↓↓
CARBO	200 mg/m²	↓↓↓ ↓↓↓ ↓↓↓ ↓↓↓
CYCLO	450 mg/m²	↓↓↓ ↓↓↓
DOX	50 mg/m²	↓ ↓
		RT
		1<----2-----3---> 4 5 6 7 8 9 10 11 12 13 14 15
		16 17 18 19 20 21 22 23 24 25 26 27
		28 29 30 31 32 33 34

Weight: ___,___ kg **Height:** ___ cm **Body surface area:** ___,___ m²

	Date	Weight [kg]	Administered dose VP16 [mg]	CARBO [mg]	CYCLO [mg]	DOX [mg]	Dose-reduction	Reason ?
We 1, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 4, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 7, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 10, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 13, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 16, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 19, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 22, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 25, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 28, d1 d2 d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 31, d1 d2 d3							☐ No ☐ Yes:	☐ Toxicity ☐ other
We 34, d1 d2 d3							☐ No ☐ Yes:	☐ Toxicity ☐ other

Version 3.0 / June 2006

F7d4 - **2/2**

Toxicity or Severe Adverse Event : ☐ No ☐ Yes (please fill in form **F8b** !)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b** !)

If patient received RT, did RT aggravate the SAE ? ☐ No ☐ Yes

Was G-CSF administered to the patient: ☐ No ☐ Yes

At the end of treatment:

☐ complete remission (CR)
☐ partial remission (PR)
☐ no change (NC)
☐ Progression (PD)
☐ unable to determine

Postoperative complication(s): _____

_____

For the documentation of cardiotoxicity please always use the form F8a

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7e - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
INITIAL SURGERY - POST-OPERATIVE CHEMOTHERAPY - REGIME 1

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

VCR 1.5 mg/m² ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓ ↓

Weeks 1 2 3 4 5 6 7 8 9 10

Stage I, intermedite risk with the exception of focal anaplasia ☐ Yes ☐ No

Weight: , kg **Height:** cm **Body surface area:** , m²

	Date	Weight [kg]	Administered VCR Dose [mg]	Dose-reduction	Reason ?
Week 1				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 2				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 3				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 4				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 5				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 6				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 7				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 8				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 9				☐ No ☐ Yes	☐ Toxicity ☐ other
Week 10				☐ No ☐ Yes	☐ Toxicity ☐ other

Toxicity or Severe Adverse Events: ☐ No ☐ Yes (please fill in form **F8b** !)

Venoocclusive diseaese (VOD): ☐ No ☐ Yes (please fill in form **F8b** !)

At the end of treatment:
- ☐ complete remission (CR)
- ☐ partial remission (PR)
- ☐ no change (NC)
- ☐ Progression (PD)
- ☐ unable to determine

Postoperative complication(s): _____

Comment(s):

Stamp: Date: Signature

Version 3.0 / June 2006

F7b - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -

INITIAL SURGERY - POST-OPERATIVE CHEMOTHERAPY - REGIME 2 (AV)

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID

VCR 1.5 mg/m²
ACT-D 45 µg/kg

Weeks 1 2 3 4 5 6 7 8 9 10 11 14 17 20 23 26

Stage I, focal anaplasia ☐ Yes ☐ No
Stage II, low or intermediate risk ☐ Yes ☐ No

Weight: ___,___ kg Height: ___ cm Body surface area: ___,___ m²

	Date	Weight [kg]	Administered dose VCR [mg]	Administered dose ACT-D [µg]	Dose-reduction	Reason ?
Week 1					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 2					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 3					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 4					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 5					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 6					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 7					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 8					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 9					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 10					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 11					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 14					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 17					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 20					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 23					☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 26					☐ No ☐ Yes: ___	☐ Toxicity ☐ other

Version 3.0 / June 2006

F7f - 2/2

Toxicity or Severe Adverse Event : ☐ No ☐ Yes (please fill in form **F8b** !)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b** !)

If patient received RT, did RT aggravate the SAE ? ☐ No ☐ Yes

Was G-CSF administered to the patient: ☐ No ☐ Yes

At the end of treatment:

- ☐ complete remission (CR)
- ☐ partial remission (PR)
- ☐ no change (NC)
- ☐ Progression (PD)
- ☐ unable to determine

Postoperative complication(s): _____

_____

For the documentation of cardiotoxicity please always use the form F8a

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7g - 1/2

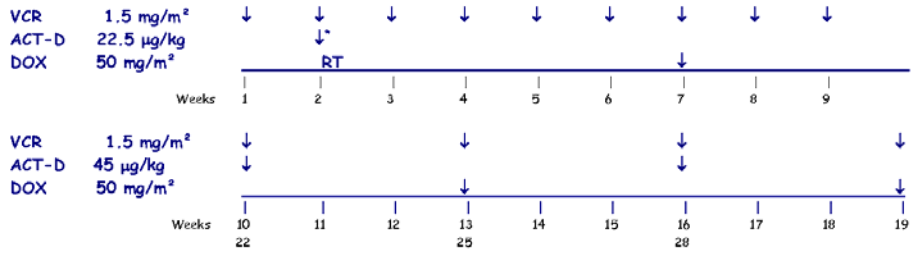
NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
INITIAL SURGERY - POST-OPERATIVE CHEMOTHERAPY - REGIME 3

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID



Stage III, intermediate risk (and focal anaplasia) ☐ Yes ☐ No

Weight: ___,___ kg **Height:** ___ cm **Body surface area:** ___,___ m²

	Date	Weight [kg]	Administered dose VCR [mg]	ACT-D [mg]	DOX [µg]	Dosereduction	Reason?
Week 1						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 2						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 3						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 4						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 5						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 6						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 7						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 8						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 9						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 10						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 13						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 16						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 19						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 22						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 25						☐ No ☐ Yes: ___	☐ Toxicity ☐ other
Week 28						☐ No ☐ Yes: ___	☐ Toxicity ☐ other

Version 3.0 / June 2006

F7g - 2/2

Toxicity or Severe Adverse Reaction : ☐ No ☐ Yes (please fill in form **F8b !**)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b !**)

If patient received RT, did RT aggravate the SAE ? ☐ No ☐ Yes

Was G-CSF given to the patient: ☐ No ☐ Yes

At the end of treatment:

- ☐ complete remission (CR)
- ☐ partial Remission (PR)
- ☐ no change (NC)
- ☐ Progression (PD)
- ☐ unable to determine

Postoperative complication(s): _____

_____

For the documentation of cardiotoxicity please always use the form F8a

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F7h - 1/2

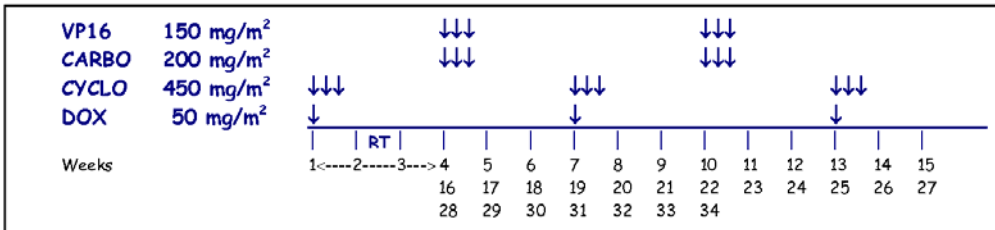
NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -
INITIAL SURGERY - POST-OPERATIVE CHEMOTHERAPY - REGIME 4 (VCD)

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no. in the study: Clinic Identification No.: (IMBEI)

Birth date

GPOH-PID



Weight: ,__ kg **Height:** ___ cm **Body surface area:** ,__ m²

	Date	Weight [kg]	Administered dose VP16 [mg]	CARBO [mg]	CYCLO [mg]	DOX [mg]	Dose-reduction	Reason ?
We 1, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 4, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 7, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 10, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 13, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 16, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 19, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 22, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 25, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 28, d1 / d2 / d3							☐ No ☐ Yes	☐ Toxicity ☐ other
We 31, d1 / d2 / d3							☐ No ☐ Yes:	☐ Toxicity ☐ other
We 34, d1 / d2 / d3							☐ No ☐ Yes:	☐ Toxicity ☐ other

Version 3.0 / June 2006

F7h - **2/2**

Toxicity or Severe Adverse Reaction : ☐ No ☐ Yes (please fill in form **F8b !**)

Venoocclusive disease (VOD): ☐ No ☐ Yes (please fill in form **F8b !**)

If patient received RT, did RT aggravate the SAE ? ☐ No ☐ Yes

Was G-CSF given to the patient: ☐ No ☐ Yes

At the end of treatment:

- ☐ complete remission (CR)
- ☐ partial Remission (PR)
- ☐ no change (NC)
- ☐ Progression (PD)
- ☐ unable to determine

Postoperative complication(s): _____

For the documentation of cardiotoxicity please always use the form F8a

Comment(s):

Stamp: Date: Signature:

Version 3.0 / June 2006

F8a - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH - CARDIOTOXICITY

Study office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Phone: (+49) 6841 16-28397 Fax: (+49) 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de

in cooperation with the German Childhood Cancer Registry at IMBEI, 55101 Mainz
Tel.: (+49) 6131/17-3227 Fax: (+49) 6131/17-4462

Name/Identification No.: Pat.-No. I-no.in the study: Clinic Identification No.: (IMBEI) Birth date

GPOH-PID

Cardiac Toxicity Scale [Common Toxicity Criteria of the NCI, Version 2.0]

CTC Grade	0	1	2	3	4
Arrhythmia	None	asymptomatic no treatment	recurr./ persist. no treatment	treatment necessary	hypotension, ventr. arrhyth. defibrillation
Cardiac function clinical	Normal	decline of resting SF or EF ≥ 10 % to < 20 %, of baseline value	decline of resting SF or EF ≥ 20% to < 25 % of baseline value	mild cardiomyopathy compensated by therapy	severe / refractory cardiomyopathy or intubation necessary
Echo (S.F.*)	≥ 30 %	≥ 25 % to < 30 %	≥ 20 % to < 25 %	> 15 % to < 20 %	≤ 15 %

	before treatment	after 100 mg/ m²	after 200 mg/m²	after 300 mg/m²	after ____ mg/m²	end of treatment
Date of examination						
CARDIAC RHYTHM						
Heart rate / min						
Arrhythmia [CTC° 1-4]						
Antiarrhythmic treatment	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
CARDIAC FUNCTION						
clinical [CTC° 1-4]						
type of toxicity						
anemia [CTC° 3 or ° 4]	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
fever [CTC° 3 or ° 4]	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
syst. / diast. blood pressure	/	/	/	/	/	/
duration of Doxorubicin inf. [h]						
echocardiography [CTC° 1-4]						
endsyst. diameter left ventricle [mm]						
enddiast. diameter left ventricle [mm]						
S.F.* value [%]						
thickness of posterior wall [mm]						
EsWS ** [g/cm²]						
septal movement (pathology)	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
pathological diastolic parameter	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
administration of Digitalis	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
ACE inhibitor therapy	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
ß-blocker therapy	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
administration of diuretics	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes	☐ Yes
LABORATORY DIAGNOSTICS						
CKMB [U/l]						
Blood to study office [Troponin, BNP, DOX-level***]	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes	☐ No ☐ Yes

* S.F.: shortening fraction; ** EsWS: end systolic wall stress; *** see form with the timepoint and the modalities of blood samp.

Signature: Date: Stamp:

Version 3.0 / June 2006

F8b - 1/2

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH

SERIOUS ADVERSE EVENTS

Study Office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: +49 6841 16-28397 Fax: +49 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
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Name/Hospital Identification No.: | Pat.-No. Identification-No.: | Clinic | Identification No.: (IMBEI) | Birthdate

GPOH-PID

Serious Adverse Events

Description and toxicity score on the second page [in right column °] :

Comment to the aetiology and nature of the SAE:

Toxicity grade according to the NCI [CTC, Version 2.0]: ☐ 3 ☐ 4

Start: **End:** **or ongoing:** ☐

Causality

Is the initial status of the patient or another illness responsible for the SAE ?

☐ yes ☐ reasonable ☐ possible ☐ improbably ☐ no

Do you believe, that the SAE is caused by the treatment ?

☐ yes ☐ reasonable ☐ possible ☐ improbably ☐ no

Please document the treatment on the corresponding forms !

Classification (Severity)

☐ Death within 4 weeks after the last treatment → **Follow-up form F9 has to be used in addition !**
☐ Life-threatening
☐ Persistend or severe late effects
☐ Hospital stay necessary or extended

Course

☐ complete recovery ☐ lacking recovery ☐ late effects ☐ death ☐ unknown

Remarks:

Stamp: Date: Signature:

Version 3.0 / June 2006

F8b - 2/2

Common Toxicity Criteria Version 2.0 of the NCI (shortened form)

Toxicity	Grade 0	Grade I	Grade II	Grade III	Grade IV	°
Blood						
Hemoglobin g/dl	WNL	> 10.0 g/dl	8.0 - 10.0 g/dl	6.5 - 7.9 g/dl	< 6.5 g/dl	
Leucocytes: 10^9/l	≥ 4.0	3.0 - 3.9	2.0 - 2.9	1.0 - 1.9	< 1.0	
Granulocytes: 10^9/l	≥ 2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	< 0.5	
Thrombocytes: 10^9/l	≥ 100	75 - 100	50 - 74.9	25 - 49.9	< 25	
Bleeding	none	Mild, no Transfusion	severe, 1 - 2 U Transf./Episode	severe, 3 - 4 U Transf./Episode	Massive > 4 U Transf./Episode	
Infection	none	mild	moderate localized infection oral antibiotics	severe systemic infection i.v.-antibiotics	life-threatening (e.g., septic shock)	
Fever without Infection	no	7.1 - 38° C	38.1 - 40° C	> 40° C < 24 hours	> 40° C > 24 h or RR ↓	
Skin	normal	Erythema	dry desquamation, vasculitis, pruritus	eruptive desquamations, ulcer	exfoliative dermatitis, necrosis	
Gastroenterology						
Bilirubin	WNL	> ULN - 1.5 x ULN	> 1.5 - 3.0 x ULN	> 3.0 - 10.0 x ULN	> 10.0 x ULN	
ALAT / ASAT	WNL	> ULN - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 - 20.0 x ULN	> 20.0 x ULN	
Alk. phosphatase	WNL	> ULN - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 - 20.0 x ULN	> 20.0 x ULN	
Stomatitis	none	painless ulcera, erythema	painful ulcera, eating possible	painful ulcera, eating not possible	total parenteral nutrition	
Cardiology SF = shortening fraction EF = Ejection fraction (left ventricular)	normal (see Colan formula for SF in children)	asymptomatic decline of resting SF or EF >10% to < 20%, control 1 week later	asymptomatic decline of resting SF or EF > 20% to < 25%, avoid next Doxorubicin	cardiomyopathy, decline of resting SF or EF > 25% treatment necessary no more Doxorubicin	severec cardiomyopathy, treatment on ICU is necessary	
Echocardiography (SF)	≥ 30 %	≥ 25 % - 30 %	≥ 20 % - 25 %	> 15 % - 20 %	≤ 15 %	
Kidney						
creatinin clearance [ml/min/1.73 m^2]	≥ 90	60 - 89	40 - 59	20 - 39	< 20	
tubulus toxicity	none	increase of β2 microglobulin and lysocyme in urine, mild hyperamino-aciduria (HAA)	decline of phosphat-reabsorbtion (TRT 75-85 %), glucosuria < 10 mmol/l, moderate HAA	Debre de Toni Fanconi syndrome, rickets, tetany, hyperchloremic metabolic acidosis, polyuria	prolonged (≥5 years) or definitive substitution or progressive kidney failure	
Neurology						
Neurocortikal function	normal	mild somnolence or agitation	moderate somnolence or agitation	mild somnolence, agitation, confusion, desorientation, hallucination	coma, convulsion, toxic psychosis	
VCR-constipation	no	mild	moderate	severe	ileus > 96 h	
Sensory function	normal	mild paraesthesia, loss of deep tendon reflexes	moderate loss of sensory functions or paraesthesia	severe loss of sensory functions or paraesthesia	permanent sensory loss	
Motoric function	normal	subjective weakness	mild but objective weakness	objective weakness with loss of function	paralysis	

Version 3.0 / June 2006

F9 - 1/1

NEPHROBLASTOMA - CLINICAL TRIAL & STUDY SIOP 2001/GPOH -

Follow-up Form

Study Office: Prof. Dr. N. Graf, Universitätsklinik für Kinder- und Jugendmedizin, 66421 Homburg/Saar
Tel: +49 6841 16-28397 Fax: +49 6841 16-28302 Email : norbert.graf@uniklinikum-saarland.de
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Name/Hospital Identification No.: | Pat.-No. Identification-No.: | Clinic | Identification No.: (IMBEI) | Birthdate

GPOH-PID

Date of last follow-up : **Treatment finished :** ☐ No ☐ Yes

Status (Response):
☐ CR ☐ PR / remaining tumour ☐ progression ☐ unchanged

Diagnosis of relapse / metastases ☐ No **if yes, CR before :** ☐ No ☐ Yes
☐ Yes, local relapse Date of relapse:
☐ Yes, metastases: Date of metastases:
☐ Lung ☐ Liver ☐ Abdomen ☐ CNS
☐ bone, where:
☐ Lymph node, where:
☐ soft tissue, where:
☐ elsewhere:
Second remission: ☐ No ☐ Yes, when:

Bilaterilisation into the contralateral kidney: ☐ No ☐ Yes

Was the patient symptomatic ? ☐ No ☐ Yes
Was the relapse / metastases diagnosed during routine follow-up ? ☐ No ☐ Yes
Was the relapse / metastases diagnosed : by clinical investigations ? ☐ No ☐ Yes
by imaging studies ? ☐ No ☐ Yes: ☐ US ☐ CT ☐ MRI
Date of last follow-up before relapse/metastases:
Date of last X-ray of the lungs before relapse/metastases:
Date of last abdominal sonography before relapse/metastases:

Second tumour: ☐ No ☐ Yes, diagnosis:
Date of diagnosis: ☐ benign ☐ malignant
Localisation:
In the irradiation field: ☐ No ☐ Yes

Late effects: ☐ No ☐ Yes : ☐ cardiac ☐ kidney ☐ skeleton
☐ else:

In case of death: date of death: Autopsy: ☐ No ☐ Yes
Cause of death:
☐ tumour ☐ treatment ☐ accident ☐ suicide ☐ else ☐ unknown

Remarks:

Stamp: Date: Signature:

Version 3.0 / June 2006

Common Toxicity Criteria Version 2.0 of the NCI (shortened form)

Toxicity	Grade 0	Grade I	Grade II	Grade III	Grade IV
Blood					
Hemoglobin g/dl	WNL	> 10.0 g/dl	8.0 - 10.0 g/dl	6.5 - 7.9 g/dl	< 6.5 g/dl
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Granulocytes: 10^9/l	≥ 2.0	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	< 0.5
Thrombocytes: 10^9/l	≥ 100	75 - 100	50 - 74.9	25 - 49.9	< 25
Bleeding	none	Mild, no Transfusion	severe, 1 - 2 U Transf./Episode	severe, 3 - 4 U Transf./Episode	Massive > 4 U Transf./Episode
Infection	none	mild	moderate localized Infection oral antibiotics	severe systemic Infection i.v.-antibiotics	life-threatening sepsis (e.g., septic shock)
Fever without **Infection**	no	7.1 - 38° C	38.1 - 40° C	> 40° C < 24 hours	> 40° C > 24 h or RR ↓
Skin	normal	Erythema	dry desquamation, vasculitis, pruritus	eruptive desquamations, ulcer	exfoliative dermatitis, necrosis
Gastroenterology					
Bilirubin	WNL	> ULN - 1.5 x ULN	> 1.5 - 3.0 x ULN	> 3.0 - 10.0 x ULN	> 10.0 x ULN
ALAT / ASAT	WNL	> ULN - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 - 20.0 x ULN	> 20.0 x ULN
Alk. phosphatase	WNL	> ULN - 2.5 x ULN	> 2.5 - 5.0 x ULN	> 5.0 - 20.0 x ULN	> 20.0 x ULN
Stomatitis	none	painless ulcera, erythema	painful ulcera, eating possible	painful ulcera, eating not possible	total parenteral nutrition
Cardiology SF = shortening fraction EF = Ejection fraction (left ventricular)	normal (see Colan formula for SF in children)	asymptomatic decline of resting SF or EF ≥10% to < 20%, control 1 week later	asymptomatic decline of resting SF or EF > 20% to < 25%, avoid next Doxorubicin	cardiomyopathy, decline of resting SF or EF > 25% treatment necessary no more Doxorubicin	severec cardiomyopathy, treatment on ICU is necessary
Echocardiography (SF)	≥ 30 %	≥ 25 % - 30 %	≥ 20 % - 25 %	> 15 % - 20 %	≤ 15 %
Kidney					
creatinin clearance [ml/min/1.73 m²]	≥ 90	60 - 89	40 - 59	20 - 39	< 20
tubulus toxicity	none	increase of ß2 microglobulin and lysocyme in urine, mild hyperamino-aciduria (HAA)	decline of phosphat-reabsorbtion (TRT 75-85 %), glucosuria < 10 mmol/l, moderate HAA	Debre de Toni Fanconi syndrome, rickets, tetany, hyperchloremic metabolic acidosis, polyuria	prolonged (≥5 years) or definitive substitution or progressive kidney failure
Neurology					
Neurocortikal function	normal	mild somnolence or agitation	moderate somnolence or agitation	mild somnolence, agitation, confusion, desorientation, hallucination	coma, convulsion, toxic psychosis
VCR-constipation	no	mild	moderate	severe	ileus > 96 h
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Motoric function	normal	subjective weakness	mild but objective weakness	objective weakness with loss of function	paralysis

Version 3.0: June 2006

III. The ACGT Breast Cancer Research

III.1 Breast Cancer Background

Carcinoma of the breast remains the most prevalent cancer diagnosed in women in the world. Although breast cancer mortality has declined in the last two decades, breast cancer continues to represent a major threat to the lives and productivity of women. The number of effective treatments for breast cancer is on the rise; however, the benefit from specific treatments to individual patients and the adverse events they experience vary considerably. Efficacy and safety of anticancer therapies may depend on tumor, treatment, and host characteristics. (1)

Small variants in the germline DNA sequence (genotype) may lead to different expression of the encoded protein or to the expression of altered protein, and thus to a different health outcome (phenotype) (2,3) The recent completion of the human genome project and advances in high throughput DNA sequencing and proteomic technologies may contribute to the understanding of interindividual variability in health outcomes. Most genetic variants occur in noncoding regions of the genome, and although such variants may result in functional consequences, most known variants that are associated with clinically important functional change are in the exons that code for protein expression (4). While the clinical importance of a large number of pharmacogenetic variants is becoming clearer, the significance of the majority remains speculative while we await larger trials. Preliminary pharmacogenetic data strongly suggest an important role for the use of germline genetic information in the individualization of treatment and prevention of breast cancer. The potential value of these data as individual genotypic predictors may be valuable or, more likely, patterns of genetic markers analogous to the expression profiles obtained from tumor tissue may allow more powerful prediction of who will respond best to a specific treatment or regimen. For the potential of genomic research to be fulfilled, prospective trials with clinical outcomes as end points will have to include the collection of germline DNA. Pharmacogenetics may play a significant role in several aspects of breast cancer including prognosis at the time of diagnosis, response to specific treatments, and likelihood of adverse events to specific treatments. While a large amount of research has examined details of genetic predisposition to breast cancer, a relative paucity of work has been done to identify genetic variants that might predict a women's prognosis and response to treatment once breast cancer is diagnosed. Indeed, once breast cancer is diagnosed, a woman may be more interested in her chances of response to treatment than her risk of carrying a genetic variant that puts her at higher risk of developing the disease(5).

When primary breast cancer has developed, certain clinical characteristics of the tumor, such as lymph node metastases, tumor size, and grade are used to predict prognosis (6). Most clinicians offer treatment recommendations to an individual woman based on estimates of likelihood of recurrence with local treatment only (prognosis), co-morbidities, and possible treatment-related toxicities. Clinicians continue to struggle to identify sensitive tools that can help separate women who are not going to suffer a recurrence from women who are likely to suffer a recurrence. Pharmacogenomics is a technically robust technology that offers considerable potential to allow better identification of subgroups of patients that may be at sufficient risk of cancer recurrence to justify systemic treatment. Much recent research has focused on the genomics of the tumor itself and the possibility that gene expression within the tumor may be predictive of risk of relapse (7,8)

Once a decision is made to administer systemic therapy, only a handful of genes or proteins are used to select specific treatments for breast cancer patients. Early results suggest that patterns of gene expression determined on primary tumors may predict sensitivity or resistance to common breast cancer treatments (9, 10) Advances in genomics may also

assist in predicting response to specific treatments. Genetic variants that might predict the composite results of treatment represented by tumor response and side effects may reside in the germline, since most tumor DNA remains the germline DNA of the patient (11) Indeed, a woman's primary breast cancer is more likely to genetically resemble her metastatic foci than another woman's primary tumor with similar histological characteristics (7). Pharmacogenomics of drug response may also be related to the genetic inheritance of single-nucleotide polymorphism (SNPs) or other changes such as insertions or deletions in important genes relevant to drug disposition and effect, including drug metabolizing enzymes, transporters, or drug targets.

Only a few predictors have been used to estimate an individual woman's risk of adverse events to specific agents or treatments. Older age, previous treatments, and co-morbid conditions may predict a higher chance of chemotherapy-related neutropenia. Germ line DNA characteristics may also play an important role in predict ing who may be at a high risk of adverse events. For example, African- American women may be at a higher risk of chemotherapy-related neutropenia resulting in treatment reductions and delays (12). Genetically predictable side effects may correlate with the expression of individual susceptibility pathways in vulnerable tissues. The prospective knowledge of an individual's risk to develop adverse events could lead to a change in the proposed treatment or to intervention that may reduce the risk.

A central, but often unrecognized goal of pharmacogenetic research, is to use the revolution in genomics to allow the benefit of treatment and avoidance of toxicity to be made available to all patients, rather than only the subgroup that can tolerate currently used therapeutic regimens and respond well to them. Knowledge of the likelihood of response to treatment and the predictability of side effects may assist in individualizing treatment for women diagnosed with breast cancer.

III.2 Post Genomic Analysis in Breast Cancer

Numerous clinical factors have been identified that are associated with patient survival, response to therapy, or both, including lymph node status, tumor size, tumor histology, age at diagnosis, and cellular proliferation rate. Several molecular markers have also been found to correlate with patient prognosis, the most important of which is expression of the ER, with ER negativity associated with poor patient survival. Although some histologic subgroups are clearly associated with specific molecular markers, for example, comedocarcinoma with ER negativity and with *ERBB2* overexpression, other such relationships are less clear; most commonly used histologic and molecular classifications fail to account for the wide histologic, molecular, and clinical heterogeneity observed in breast cancers. Considerable effort over the last few years has gone into elucidating the genetics, biology, pathology, and clinical outcome of breast cancer using high-throughput gene expression profiling methods.

III.2.1 Discovery of Disease Subclasses

Some of the earliest studies using global gene expression profiling dealt with the classification of breast cancer into subgroups representing breast tumors with similar transcriptional profiles. In a study of invasive ductal breast carcinomas, investigators used unsupervised analyses, identifying five distinct subtypes based on gene expression profiling.These analyses largely separated breast tumors into two main groups: those positive for the ER expression (ER+) and those negative for ER (ER–). This finding has since been recapitulated in several other studies, suggesting that ER status makes the strongest impact on the gene expression patterns of breast cancers and reinforcing the fundamental role of ER in the development and progression of breast cancer. The unsupervised analyses further subdivided the ER+ and ER– groups into unique subgroups with differences in patient survival. ER+ tumors, which are characterized by expression of several molecular markers of

normal luminal epithelial cells, can be further divided into two or three smaller "luminal" subgroups. Patients who comprise the luminal subgroup defined by the highest expression levels of luminal/ER-associated genes have a poorer 5-year survival after adjuvant therapy than those with low to moderate expression of these genes.The ER– group was likewise divided into three subgroups, characterized by expression of markers of adipose-enriched normal breast tissues, markers of normal breast basal epithelial cells, or high-level expression of the oncogene *ERBB2*, respectively. The suggestion that ductal breast carcinomas can be derived from two distinct cell types (basal or luminal) is intriguing, especially in the light of suggestions of the presence of a breast stem cell, and warrants further investigation. The clinical significance of these proposed novel subgroups remains an open question; however, the prognostic heterogeneity suggested in these studies illustrates the need for more targeted treatment regimens for subsets of patients with breast cancer and also demonstrates the potential for gene expression profiling in identifying these subgroups.

III.2.2 Predicting Clinical Outcome

Currently available criteria used to predict disease progression and clinical outcome in breast cancer, including tumor size, age at diagnosis, lymph node status, histologic grade, and ER status, are imperfect, and, consequently, improved tools are needed for the assessment of prognosis and treatment prediction in breast cancer. The first steps toward associating gene expression patterns with survival in breast cancer have been reported. In a study comprised of lymph node–negative breast cancer patients who did not receive adjuvant therapy, a supervised analysis was used to identify a genetic signature consisting of 70 genes that distinguished between patients who developed metastases within 5 years from those who did not. A follow-up study performed by the same investigators, including lymph node–positive and –negative breast cancer patients, used the expression levels of these same 70 genes to assign them to good- and poor-prognosis groups that differed significantly in the rate of metastasis development and survival. Interestingly, the prognostic profile did not seem to correlate with lymph node status but was indeed associated with the age of the patient at diagnosis, the histologic grade of the tumor, and ER status—three of the most commonly used prognostic factors in breast cancer. In keeping with the previously mentioned key role of ER in breast cancer classification and outcome prediction, the vast majority of tumors within the good-prognosis group were ER positive.

The use of gene expression profiling for prognostic purposes illustrates that the molecular signatures of tumors contain information regarding clinical behavior. The prognostic studies performed to date, however, are extremely small in the context of evaluation of prognostic indicators and have only been applied to a subset of breast cancer patients with less advanced disease. Clearly, prospective studies based on larger patient cohorts representing the whole spectrum of breast cancer are needed before gene expression profiling can be introduced into the routine clinical setting. Nevertheless, these initial studies have been promising enough to justify a clinical trial in which this array-based diagnostic will be used to guide decisions as to whether patients will receive adjuvant therapy after surgery. Further studies aimed at elucidating the effect of different treatment regimens on disease outcome, combined with efforts to develop targeted therapies, are needed to identify those patients who are most likely to benefit from available and novel adjuvant treatments.

III.2.3 Use of Microarrays to Detect Familial Predisposition Genes

Approximately 5% to 10% of breast cancers are of hereditary origin, and two major breast cancer susceptibility genes have been identified to date, *BRCA1* and *BRCA2*.Mutation screening in these two genes for hereditary breast cancer families has become commonplace at oncology clinics across the world, allowing gene carriers to make informed decisions regarding intensive surveillance programs, prophylactic treatments, or both. The techniques

used for screening are, however, time consuming, laborious, and expensive, especially because both genes are large and mutations are spread across the entire coding region. Although *BRCA1*-derived breast cancers display certain histopathologic characteristics that may aid in the characterization of *BRCA1* tumors, these tumors do not constitute an entirely uniform group. Moreover, *BRCA2* breast cancers make up a considerably more heterogeneous group. An alternative means of identifying *BRCA1*- or *BRCA2*-associated tumors would greatly facilitate the identification of patients who carry mutations in these genes, particularly those with an unknown family history. Finally, extended knowledge of the defect(s) causing the development of breast cancer may greatly improve treatment schemes and intervention strategies for the affected individuals.

Investigations of gene expression profiles in hereditary breast cancers have illustrated that tumors derived from individuals with *BRCA1* mutations can be distinguished from those with *BRCA2* mutations based on gene expression profiles. This finding could have clinical implications in that it may become possible to perform gene expression profiling analyses based on a set of highly informative genes (a *BRCA1/2* diagnostic gene chip) to determine if a potential mutation carrier belongs to the *BRCA1* or *BRCA*2 group. The most intriguing finding in this study was the discovery of a *BRCA1*-like gene expression profile in a tumor from a patient without a germline mutation in the gene. Instead, the promoter of the *BRCA1* gene showed aberrant methylation resulting in silencing of gene expression, and this was found to be the underlying cause of the *BRCA1*-like gene expression profile of the tumor. Because epigenetic events such as promoter methylation can be important in tumorigenesis, this finding points to the use of expression profiling for identifying such events in the absence of germline alterations. It also illustrates the high degree of sensitivity of transcriptional profiling and demonstrates that defects in individual genes give rise to unique and characteristic genetic changes that may, on further investigation, shed light on the functional relationship between specific genetic or epigenetic defects and disease.

Although *BRCA1* and *BRCA2* were initially proposed to be responsible for the majority of inherited breast cancer, more recent population-based studies suggest that they account for a far smaller portion of familial breast cancer, with considerable variation between different populations. Presumably, non-*BRCA1/2* (*BRCAx*) hereditary breast tumors may arise as a result of mutations in other high-penetrance genes, or perhaps as a result of low-penetrance alleles (e.g., *CHEK2*). The non-*BRCA1/2* subgroup of breast cancer appears to comprise a histologically heterogeneous group, indicating the presence of multiple underlying alterations. The heterogeneity of cancer-predisposing mutations in *BRCAx* families has severely limited the power of traditional linkage analysis. With no current means to identify subgroups of *BRCAx* families with cancer-predisposing mutations in a common gene, the search for new breast cancer predisposition genes has been confounded. Although in many cases frozen tumor material is unavailable, studies have demonstrated the power of expression profiling for this purpose. Familial *BRCAx* tumors can indeed be subclassified into homogeneous subsets, separate from *BRCA1* and *BRCA2* tumors, based on gene expression patterns. Furthermore, copy-number analysis of genomic DNA from these same tumors using microarray-based comparative genomic hybridization (CGH) revealed that these subgroups were each associated with specific somatic genetic alterations, further supporting the hypothesis that there are multiple distinct subclasses of *BRCAx* tumors. These findings illustrate that gene expression–based profiling can be used to identify distinct and homogeneous subclasses within the non-*BRCA1/2* (*BRCAx*) familial breast cancers, and microarray-based CGH can be used to identify distinct chromosomal aberrations within these subgroups, thereby potentially increasing the power of conventional genetic analysis by enabling the search for novel breast cancer genes within homogeneous subsets of families.

III.3 Pharmacogenetics of Systemic Breast Cancer Treatments

Systemic treatments for breast cancer are divided into hormonal interventions, chemotherapy, and novel agents.Antitumor activity or safety of specific agents may depend not only on drug dose and schedule but also on functional targets, drug metabolizing enzymes, and transporters. Some agents are prodrugs with one or more metabolites that may contribute to the drug's antitumor activity or to specific side effects. Prospective determination of genetic variants in drug metabolizing enzymes or drug transporters could be used to determine likelihood of response and/or propensity to adverse effects. Response to a specific agent may also depend on variants in the target of the treatment. It is possible that small genetic variations in the target may affect the response or toxicity related to the agent. In this review we will focus on the current knowledge of the role of pharmacogenetics in predicting efficacy and safety of standard and emerging breast cancer treatments.

III.3.1 Hormonal Therapy

More than 50% of primary breast cancers will express the estrogen receptor (ER) and/or progesterone receptor (PgR). Almost every woman with hormone receptor-positive disease will be offered some form of hormonal intervention to treat the cancer. Most women with early breast cancer will likely receive adjuvant tamoxifen for 5 years. Postmenopausal women may be offered aromatase inhibitors instead of or following tamoxifen, and premenopausal women may undergo ovarian suppression instead of or with tamoxifen. Tamoxifen has also been approved to reduce the incidence of a new breast cancer in women at high risk for the disease.

III.3.2 Chemotherapy

Several single agent and combination chemotherapy regimens are effective treatments for breast cancer. In metastatic disease the goal of therapy is to improve time to progression, quality of life and possibly overall survival. Cure is an attractive goal but is rarely achieved in this setting. In contrast, in the adjuvant setting, the ultimate goal is to improve overall and/or diseasefree survival. The optimal dose and schedule of chemotherapy are generally determined in phase I clinical trials, usually in the metastatic setting, when dose-limiting toxicities are assessed and a maximum tolerated dose is determined. Then, the dose and schedule may be further refined in phase II and III trials. Promising drugs are then tested in the adjuvant setting. Most chemotherapy drugs are administered to an individual based on a body surface area (BSA) calculated from the patient's height and weight or, less often, area under the curve (AUC). Currently, there is no consensus regarding how to dose people who are not at their ideal body weight, whether adjustments should be made based on age and toxicity, or lack of it, to the treatment, or whether BSA should be used to determine dose of chemotherapy and novel treatments. (13).

Many women and their healthcare professionals will accept modest drug related toxicity for modest improvement in outcome. However, decision making is especially difficult for women with very small tumors that are likely cured of their cancer by local modalities who may gain little benefit, if any, from the addition of adjuvant chemotherapy. If we were able to quantify the benefit an individual woman may derive from a specific treatment and her risk of developing serious, life-threatening, or long term toxicities, she could then weigh the specific benefits and possible adverse events to make a decision regarding treatment. This key information could make a risk and benefit discussion more personal and less theoretical.

Table 1. Common chemotherapy agents administered to women with breast cancer (1)

Regimen	Mechanism of action	Administration	Common toxicities
Cyclophosphamide	*Alkylating agent. Requires metabolic activation by cytochrome P450 enzymes to 4-hydroxy-cyclophosphamide to exert antitumor activity*	*Commonly administered in combination with methotrexate and 5-fluorouracil (CMF) or with anthracyclines. A common component of myloablative regimens rarely used in breast cancer*	*Myelosuppression, mainly leukopenia, nausea and vomiting, skin and nail hyperpigmentation, gonadal dysfunction. High dose: hemorrhagic cystitis, secondary leukemia*
Doxorubicin, epirubicin	*Anthracyclines. Form complexes with DNA by intercalation between base pairs, leading to formation of free radicals and inhibition of DNA topoisomerase II catalytic activity. Activates protein kinase C-mediated signal transduction pathways*	*May be administered as single agent or in combination with cyclophosphamide with or without 5-fluorouracil. Newer combination contain paclitaxel or docetaxel*	*Myelosupression, mainly leukopenia, acute and delayed nausea and vomiting, mucositis, and skin and nail hyperpigmentation. Acute and chronic cardiac toxicity associated with higher cumulative doses or with other predisposing cardiac factors.*
Paclitaxel, docetaxel	*Promotes microtubule assembly and stabilizes tubulin formation to induce mitotic block. Possible proapoptotic and antiangiogenic activity*	*Commonly administered as single agent. May be administered with anthracyclines. Promising new combinations include docetaxel and capecitabine, paclitaxel or docetaxel and gemcitabine*	*Myelosuppression, mainly leukopenia, myalgias and arthralgias, sensory peripheral neuropathy, and sporadic anaphylactoid reactions (greatly reduced by the administration of corticosteroids). Edema and nail changes may be seen with higher cumulative doses of docetaxel.*
5-Fluorouracil, capecitabine	*Analogue of naturally occurring pyrimidine uracil. Inhibit thymidylate synthase (TS), which has a significant role in catalyzing deoxyuridylate (dUMP) to thymidilate (dTMP)*	*5-Fluorouracil may be administered in combination with methotrexate and cyclophosphamide (CMF) or with anthracyclines. Continuous infusion of 5-fluorouracil has been commonly used as third-line therapy, now replaced by oral capecitabine*	*Myelosuppression, nausea and vomiting, diarrhea, and hand foot syndrome. Rare ocular and neurological toxicity*

In adjuvant breast cancer, combination chemotherapy has been the standard of care for many women for decades. Combinations of cyclophosphamide and/or anthracycline-based therapy are usually administered. Some common agents administered in breast cancer are summarized in Table 1 (1) In metastatic disease, single agent therapies are often administered although several combinations have also been used. Several tumor characteristics, such as poor grade and lack of hormone receptors, have been associated with improved response to chemotherapy; however, specific predictors of response to individual chemotherapy agents are not yet identified. Likewise, pharmacogenetic factors have been suggested but not proven to predict toxicity to treatment.

Inherited variation in the activity of drug-metabolizing enzymes that handle some chemotherapeutic agents is well recognized. This variation may result in interindividual differences in pharmacokinetics of specific agents and may be associated with treatment-related toxicity. For example, it is well established that people with the rare recessive deficiency of dihydropyrimidine dehydrogenenase enzyme (DPD), which reduces 5-fluorouracil to an inactive metabolite, dehydrofluorouracil, will have delayed clearance of the drug and therefore greatly prolonged half-life in the plasma. Due to the long exposure to the parent drug, people with DPD deficiency will suffer increased toxicity, especially neurotoxicity. Knowledge of pharmacogenetic variables that may influence response or risk for adverse events is important both in the metastatic and adjuvant treatment of breast cancer. If we could select the treatment that is most likely to provide benefit and minimal toxicity as first line therapy, we may improve long-term outlook.

Chemotherapy agents that are commonly used in breast cancer as well as potential pharmacogenetic factors that may affect their use are reviewed (Table 2, next page) (1). Only a few small retrospective analyses have been conducted and germline DNA samples have rarely been collected in prospective trials to examine pharmacogenetic effects on response or toxicities to chemotherapy in breast cancer.

⇨ ***Cyclophosphamide (Cytoxan).*** Specific drugs used in breast cancer chemotherapy regimes have evolved over the years, but cyclophosphamide has remained a stable component in many of the combinations. Cyclophosphamide (Cytoxan) is rarely used as a single agent in breast cancer and thus it is difficult to ascertain whether the efficacy or toxicity seen in combination treatments is related to cyclophosphamide or other agents in the combination. Cyclophosphamide is also an integral component of several high-dose chemotherapy combina tions, an approach rarely utilized in breast cancer at present. Cyclophosphamide is a mechlorethamine- analog, with activity against many tumors. It is a pro-drug that requires metabolic activation by cytochrome P450 enzymes to 4-hydroxycyclophosphamide before it exerts its effects.(14) 4-Hydroxy-cyclophosphamide equilibrates with aldophosphamide, and the latter can undergo chemical decomposition into phosphoramide mustard and acrolein. Phosphoramide mustard is an active DNA alkylating metabolite. Acrolein is a toxic byproduct, which has been implicated as the cause of hemorrhagic cystitis, illustrating the significance of drug metabolism and consequential adverse effects. Aldophosphamide can also be metabolized into the inactive metabolites carboxyphosphamide and 4-ketocyclophosphamide.

Multiple cytochrome P450 enzymes have been implicated in the metabolic activation of cyclophosphamide, including CYP2A6, CYP2B6, CYP2C19, CYP2C9, CYP3A4, and CYP3A5,(15–18) but the relative importance of each of these enzymes in the treatment of breast cancer with cyclophosphamide remains unknown. Of these, CYP2B6, CYP2C19, CYP2C9, and CYP3A5 have known variant alleles that influence their expressed proteins, and may be associated with altered metabolic activity and result in the wide interpatient variability that is seen in cyclophosphamide-treated individuals. (19,20) It is possible that genetic differences in cytochrome P450 enzymes may increase the likelihood of myelosuppression and/or other cyclophosphamide related toxicities. If we could screen for genetic predisposition to drug-related toxicity, we could offer vulnerable women prophylactic treatments, such as the use of colony stimulating growth factors to prevent myelosuppresion. Glutathione-S-transferase (GST) detoxifies mutagenic and cytotoxic DNA-reactive metabolites. GST may be duplicated, deleted, or mutated, and these genetic changes may alter the function of the enzyme. Deleted or mutated GST may be associated with less detoxification of cyclophosphamide, resulting in more available drug compared to the wild-type enzyme. In a retrospective analysis of women who received adjuvant cyclophosphamidebased regimens, polymorphic GST (Ile105Val) was indeed associated with improved overall survival compared to women with wild-type enzyme.(21)*.*

Genetic predisposition may also be an important predictor for long term and potentially serious toxicities of chemotherapies. Cyclophosphamide and other alkylating agents have been implicated in the risk for secondary leukemia, especially when administered in high dose. Although direct correlation between genetic variability and risk of cyclophosphamide-associated leukemia is not available, investigations in other cancers suggest that treatment-related leukemias are most often seen in patients with rare cancer predisposition syndromes or in those with polymorphisms in drug metabolizing enzymes that may result in impaired detoxification of the agents or inefficient repair of agentinduced damage.(22, 23) Identification of possible candidates may be important to gauge risk for such devastating long-term effects. This is especially important for women who may have only a small benefit from the chemotherapy.

Table 2. Possible polymorphisms that may influence efficacy or safety of common breast cancer treatments (1)

Drug	Genes*	Role of gene	Events**
Tamoxifen	**ER**	*Target*	*Primary resistance, acquired resistance, tamoxifen-stimulated growth*
			May affect other tamoxifen benefits/risks
	CYP2D6	*DME*	*Diminished concentrations of 4-hydroxy-N-desmethyl-tamoxifen (endoxifen); effect on efficacy or safety unknown*
	SULT1A1	*Elimination of active metabolites*	*Two-fold lower sulfation of the antiestrogenic metabolite 4-hydroxy-tamoxifen*
Aromatase inhibitors	**CYP19 (aromatase)**	*Target*	*May be associated with primary resistance May affect drug-related toxicity*
	CYP1A2 **CYP2C9** **CYP3A**	*DME*	*Unknown*
Cyclophosphamide	**GST**	*Detoxifies DNA-reactive metabolites*	*Improved outcomes due to lower enzyme activity and greater drug availability*
Methotrexate	**MTHFR**	*Regulate the pool of intracellular folates available for nucleic acid and protein synthesis*	*Lower activity of the enzyme results in retention of folates, possibly increasing bone marrow sensitivity to the drug*
Doxorubicin	**GST**	*Detoxifies DNA-reactive metabolites*	*Improved outcomes due to lower enzyme activity and greater drug availability*
	MDR	*Transporter*	*May correlate with resistance*
Epirubicin	**UGT2B7**	*Inactivation*	*Unknown*
Paclitaxel	**CYP2C8**	*DME*	*May correlate with reduced metabolism of paclitaxel May correlate with resistance*
	MDR	*Transporter*	*Unknown*
Docetaxel	**CYP3A4** **CYP3A5**	*DME*	*DME*
	MDR	*Transporter*	*May correlate with resistance*
5-Fluorouracil, capecitabine	**DPD**	*DME*	*Worse toxicity, especially neurotoxicity, death*
	TS	*Target*	*Increased expression correlated with worse outcomes*

***** Genes with described variants that may effect drug efficacy or safety

****** Possiple events due to genetic polymorphism

DME—drug metabolizing enzyme; DPD—dihydropyrimidine dehydrogenenase; ER—estrogen receptor; GST—glutathione-5-transferase; MDR—multidrug resistance; MHTFR—methylenetetrahydrofolate reductase; SULT1A1—sulfotransferase 1A1; TS—thymidylate synthase; UGT—UDP-glucuronosyltransferase.

⇨ ***Methotrexate.*** While single-agent methotrexate is not a common approach for the treatment of breast cancer, one of the oldest and most widely used regimens is CMF (cyclophosphamide, methotrexate, 5-fluorouracil). Methotrexate inhibits dihydrofolate reductase, resulting in partial depletion of reduced folates. The regimen is fairly well tolerated by most women, but a small portion may suffer acute myelosupression. Investigators have hypothesized that polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene may result in differential toxicity to CMF. MTHFR regulates the pool of intracellular folates that are available for nucleic acid and protein synthesis by converting 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, which is a methyl donor in the conversion of homocysteine to methionine during protein synthesis.

An SNP in exon 4 of the MTHFR gene (C677T) may result in protein with a lower activity, (24) altering the distribution of intracellular folates and leading to retention in folates. Breast cancer patients with the TT genotype may be more sensitive to bone marrow toxicity during CMF treatment. In a small retrospective analysis, TT genotype was detected in five of six patients who suffered severe toxicity to the first cycle of CMF, compared to a 20% prevalence in women who did not suffer unusual toxicity. (25)

⇨ ***Taxanes*.** Paclitaxel (Taxol) is a novel chemotherapy agent derived from the bark of the yew tree (Taxus Brevifolia) that promotes microtubule assembly and stabilizes tubulin formation. Docetaxel (Taxotere) is a semisynthetic analog of paclitaxel. The taxanes inhibit proliferation by inducing mitotic block; however, the drugs may have other properties including antiangiogenic effects that have not been entirely elucidated.

Paclitaxel is metabolized by CYP3A4 to a minor active metabolite and by CYP2C8 to a major inactive metabolite 6-8-hydroxpaclitaxel. Paclitaxel is also a P-glycoprotein substrate. Several CYP2C8 polymorphisms have been identified including CYP2C8*3, CYP2C8*4, and CYP2C8*2. In vitro, CYP2C8*3 and, to a lesser extent, CYP2C8*4 were associated with diminished catalytic activity of the conversion of the parent drug to 6-ahydroxytaxol compared to the wildtype genotype. (26) CYP2C8*2 is associated with a lower clearance of paclitaxel compared to the wild-type allele. (27) Whether this change in metabolism may translate to an alteration in paclitaxel's efficacy and/or toxicity is not known. Pharmacodynamic pharmacogenetics may also play a role in paclitaxel efficacy, but in a study of 82 women with breast cancer who were treated with paclitaxel, mutations of class I b-tubulin were detected within 18% of the tumors and were not associated with a differential response to the drug.(28)

The clearance of docetaxel is decreased with older age, decreased BSA, increased levels of albumin, and elevated bilirubin or transaminases.(29) Docetaxel undergoes hydroxylation by CYP3A4 and CYP3A5. Like paclitaxel, docetaxel is a substrate of Pglycoprotein.

A great interindividual variability in the activity of CYP3A has been observed in humans.(30) In vitro, CYP3A activity in hepatic tissues is also variable.(31) Investigators have hypothesized that it is possible to assess the activity of CYP3A4 by analyzing metabolism of erythromycin and then optimize the dose of docetaxel that can then be determined for an individual.(32) In a study of 21 patients with heavily pretreated sarcoma with good hepatic function who received docetaxel 100mg/m2, low erythromycin breath test results correlated directly with reduced docetaxel clearance.(31) The patients with the worse toxicities were indeed the patients with the lowest erythromycin breath test results and docetaxel clearance. The same investigators have subsequently used baseline erythromycin breath test results to determine the optimal dose of docetaxel for women with breast cancer. In a preliminary manner, the lowest docetaxel dose administered (which by definition correlates with lowest erythromycin breath test results) was associated with the highest AUC of the drug while the patients who received the highest dose of the drug had the lowest AUC.(33) Whetherthis noveldosing algorithm will translate to an improved efficacy or toxicity for individual patients is not known.

The importance of CYP3A5 in docetaxel metabolism has not been defined. Common polymorphisms in CYP3A5 may result in altered hepatic clearance of several drugs such as the commonly prescribed erythromycin. Thus, it is possible that an individual with a polymorphic CYP3A5 may require less than the standard prescribed dose of the drug compared to an individual with a wild-type enzyme. (34) Only 30% of Caucasians express the enzyme; however, CYP3A5 expression, and thus perhaps its significance, is higher in African Americans. (35) An SNP in the third intron of CYP3A5 occurs commonly and results in a truncated nonfunctional enzyme. The CYP3A5*3 (A22893G) allele has been correlated with alternative splicing and a truncated protein and is the most common

reason for loss of expression of the enzyme in hepatic tissue. The CYP3A5*6 polymorphism (A30597G) results in deletion of exon 7 and a low CYP3A activity. Docetaxel metabolism may correlate with an aggregate expression of CYP3A enzymes. (36)

- ***5-Fluorouracil and Capecitabine*.** 5-Fluorouracil has been used in the treatment of many solid tumors for several decades. The newer oral formulation, capecitabine (Xeloda), is also widely used. 5-Fluorouracil is reduced to the inactive metabolite dehydro-fluorouracil via DPD enzyme. DPD activity may be deficient in 3–5% of individuals, but a complete deficiency is extremely rare. (37) Tumors with low expression of DPD mRNA and activity are associated with improved response to fluorouracil and improved survival. (38) Over 20 functional mutations have been reported in the DPD gene, and it is possible that more than one gene mutation is required to predict for the lack of function of the enzyme and risk of severe toxicity and death. Thus, the clinical utility of genetic testing for specific mutations is unknown. (39) Despite the known pharmacogenetic correlate between DPD deficiency and toxicity, clinicians have rarely determined this pharmacogenetic predisposition prior to treatment recommendations.

  Thymidylate synthase (TS), the target of fluorouracil, and a folate-dependent enzyme, play an important role in cellular expression of several genes and can affect cell proliferation and death.(40) Thus, a change in the expression or function of the gene may result in altered chemosensitivity. Specifically, TS catalyzes deoxyuridylate (dUMP) to thymidilate (dTMP). dTMP is then metabolized to dTTP and incorporated in DNA synthesis. The TS gene has been shown to have different numbers of tandem repeat sequences (2, 3, 4, and 9 respectively in TSER*2, TSER*3, TSER*4, and TSER*9) that cause a differential activity by causing increased expression which is correlated with worse response. (41–43) A polymorphism in the tandem repeat sequence of the TS gene is associated with improved response and survival of colorectal cancer patients. (44) Prospective investigations of this type in patients with breast cancer receiving capecitabine are ongoing.

- ***Anthracyclines*.** The anthracycline antibiotic doxorubicin (Adriamycin) is one of the most commonly used chemotherapy agents in breast cancer. Doxorubicin forms a complex with DNA by intercalation between base pairs, leading to formation of free radicals and subsequent inhibition of DNA topoisomerase II catalytic activity. In addition, the drug activates protein kinase C-mediated signal transduction pathways. Doxorubicin is metabolized in the liver via cytoplasmic aldoketoreductases to an active metabolite, doxorubicinol and via CYP450 enzymes to inactive doxorubicinol and 7-deoxydoxorubicinone metabolites. Doxorubicin is also a substrate of P-glycoprotein, the product of the MDR-1 gene. (45) Several candidate gene polymorphisms may be important in doxorubicin efficacy or toxicity. In vitro, GST and the MDR-1 may contribute to doxorubicin resistance. (46) As noted, GST catalyzes reduction of products that result from reactive oxidant damage to DNA and lipids, such as metabolites of the chemotherapy agents, cyclophosphamide and doxorubicin. In a retrospective analysis of 251 women who received combination CAF (cyclophosphamide, doxorubicin, 5-fluorouracil), those homozygous for the deletions GSTM1*0 and GSTT1*0 were less likely to suffer disease recurrence and/or death compared to those with wild-type enzyme. (47) Genetic variants that influence the activity of this multidrug transporter have been shown to influence the disposition of a number of drugs.51 Whether the doxorubicin metabolism pathway or individual metabolites may be responsible for the toxicity associated with the drug is not known. In a study of 68 women who received preoperative anthracycline with or without taxanes, response rates were greater for those with wild-type MDR-1 compared to the TT genotype in exon 26. (48)

Epirubicin is the 40 isomer of doxorubicin, and its clearance correlates significantly with hepatic function. Epirubicinmay be less cardiotoxic than doxorubicin perhaps because it is handled differently by the liver, via a glucuronidation route that is available to epubicin and epirubicinol. Indeed the formation of epirubicin glucuronide by liver UDP-glucuronosyltransferase (UGT) is its main inactivating pathway. The glucuronide metabolites of epirubicin and epirubicinol are not active, but could divert epirubicin from free radical formation, which may induce cardiotoxic effects. This may explain, at least in part, a lower cardiotoxicity of this new anthracycline relative to that of the progenitor. Ratain et al have determined that the specific glucuronidating enzyme of epirubicin is UGT2B7,(49) a genetically polymorphic enzyme. However, genetic variants that influence epirubicin metabolism in vivo have not been described.

- ***Novel agents.*** Many novel therapies that target a specific protein or process are under preclinical and/or clinical investigation. Specific treatments may target processes such as signal transduction, antiangiogenesis, invasion, and metastases. ***Trastuzumab*** represents an example of an effective targeted therapy that is efficacious as a single agent or in combination with chemotherapy in women whose tumors overexpress or amplify c-erbB-2 or HER-2/neu. Resistance to trastuzumab may be associated with more specific tumor characteristics such as the need for an activated HER2 or proliferation of non-HER2 expressing cells. Polymorphisms in the HER2 receptor have been evaluated in context of breast cancer risk. Pharmacogenetic effects of the target or enzymes that may metabolize trastuzumab and may predict response or toxicity to the treatment have not been studied.

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III.4 The ACGT - Trial of Principle (ACGT-TOP) Study

III.4.1 The ACGT - TOP Study Research Teams

III.4.1.1 The Jules Bordet Institute Research Team (ACGT partner No: 6)

Department - Roles	**Persons** (involved)
Medical Oncology	V D'Hondt, M Piccart, C Sotiriou, F Cardoso, C Bernard, A Awada, T Gil, A Di Leo.
Surgery	J-M Nogaret, D Hertens, I Veys, D Noterman
Radiotherapy	C Philippson
Radiology	C Vanhaudenaerde
Pathologist	D Larsimont
Biological markers evaluation	V Durbecq, C Desmedt, G Rouas
DNA micro-arrays	C Sotiriou
Statistics	M. Buyse *(International Institute for Drug Development, Brussels)*
Data Management	S Dolci, A Huart, J-Y Leroy
Trial logistics, implementation, monitoring and coordination	C Desmedt

III.4.1.2 The University of Crete & FORTH Research Team

Department - Roles	**Persons** (involved)
Medical Oncology	V. Georgoulias, D. Mavroudis (UoC; ACGT partner No: 13)
Surgery	D. Tsiftsis, E. Sanidas, J. Askoxylakis (UoC)
Radiotherapy	S. Koukouraki (UoC)
Radiology	E. Contoyannis (UoC)
Pathologist	S. Stathopoulos, M. Kafousi (UoC)
Biological markers evaluation	S. Stathopoulos, M. Kafousi (UoC), D Kafetzopoulos (FORTH/IMBB)
DNA micro-arrays	D. Kafetzopoulos (FORTH/IMBB; ACGT partner No: 2)
Data Management	G. Potamias, A. Analyti, M. Tsiknakis (FORTH/ICS; ACGT partner No: 2)
Trial logistics, implementation, monitoring and coordination	E. Sanidas (UoC0, D. Kafetzopoulos (FORTH/IMBb)

III.4.1.3 Other Collaborating Centres Outside ACGT

BELGIUM	Docteur Vincent Richard Clinique Louis Cathy Rue Louis Caty, 136 7331 Baudour +32 65 76 83 73 Richard.Vincent@belgacom.net	Docteur Martine Thirion CHU Ambroise Paré Boulevard Kennedy, 2 7000 Mons +32 65 39 29 75 martine.thirion@tiscali.be	Docteur Jean-Pierre Kains HIS - Site Etterbeek-Ixelles Rue Jean Paquot 63 1050 Bruxelles + 32-2-641 48 51 jpkains@his.irisnet.be
	Docteur Frederic Forget Centre Hospitalier de l'Ardenne Avenue d'Houffalize, 35 6800 Libramont +32 61 23 81 11 *Frederic.Forget@CHA.BE*	Docteur Françoise Majois Hôpital de Jolimont 159, Rue Ferrer 7100 Haine Saint Paul + 32 64 23 30 11 francoise.majois@skynet.be	Docteur Marie Marevoet Clinique Saint-Pierre Avenue Reine Fabiola, 9 1340 Ottignies + 32 10 43 72 11
	Docteur Guy Jerusalem Bâtiment B23 Sart Tilman 4031 Angleur 4000 Liège + 32 43 66 72 01 g.jerusalem@chu.ulg.ac.be	Docteur Joseph Kerger Cliniques Universitaires de Mont Godinne Avenue du Dr Therasse, 1 5530 Yvoir + 32 81 42 21 11	Docteur Anita Vindevoghel Clinique Ste Elisabeth Place Louise Godin, 15 5000 Namur + 32 81 72 04 11
LUXEMBOURGH	Docteur Caroline Duhem Centre Hospitalier de Luxembourg Hematology-Oncology Department Rue Barblé 4 L 1210 Luxembourg Luxembourg +352 44 11 20 84 duhem.caroline@chl.lu		
FRANCE	Docteur Suzette Delaloge Institut Gustave Roussy Rue Camille Desmoulins 94 800 Villejuif + 33 1 42 11 51 27 delaloge@igr.fr	Docteur Thierry Petit CLCC Paul Strauss Rue de la Porte de l'Hôpital, 3 67 085 Strasbourg + 33 3 88 25 24 24 tpetit@strasbourg.fnclcc.fr	
CYPRUS	Docteur Yiola Marcou Oncology Centre Nicosia Acropoleos Avenue, 32 2006 Strovolos - Nicosia + 357 22 84 13 00 yiola.marcou@bococ.org.cy		

Study Coordination & Correspondence

Dr V. D'Hondt
125, bd de Waterloo (3rd floor)
1000 Bruxelles
Tel: +32-2-541 31 96
Fax: +32-2-538 08 58
Email: veronique.dhondt@bordet.be

III.5 Rationale for the ACGT-TOP Study

III.5.1 Rationale for early breast cancer

III.5.1.1 Benefits and risks of adjuvant chemotherapy for early breast cancer

Three to six months of adjuvant chemotherapy (CT) with CMF (cyclophosphamide-methotrexate-5-fluorouracil) or an anthracycline-based regimen is associated with highly significant 15-year absolute reduction in death for young women (< 50) with node negative (7%) and node-positive (11%) breast cancer, and for postmenopausal women with node negative (2%) and node-positive (3%) breast cancer, regardless of the added use of Tamoxifen (1, 2).

The most common and acute dose-limiting hematological toxicity seen with adriamycin and epirubicin is reversible leucopenia and/or neutropenia, although anemia and thrombocytopenia can also occur. Non-hematological toxicities include: alopecia, nausea and vomiting, diarrhea and stomatitis, and cutaneous and hypersensitivity reactions. All these toxicities are acute, reversible and usually manageable, particularly with the advent of new anti-emetic drugs.

Of greater concern are two possible long-term toxicities, namely cardiotoxicity and secondary leukemia.

The long-term hematological disorders most commonly associated with anthracycline-based chemotherapy (CT) are acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS). The cumulative risk, at five years, of developing treatment-related leukemia is in the range of 1% for both drugs, and is directly related to the administered dose.

Late cardiac toxicity associated with anthracycline-based CT is of low frequency (±1%), but is a potentially serious and even life-threatening event. It varies from asymptomatic drop of left ventricular ejection fraction (LVEF) to clinically relevant congestive heart failure (CHF) and correlates directly with the cumulative dose administered, rising exponentially for doses of doxorubicin above 500 mg/m² and epirubicin above 900 mg/m². Other associated risk factors include cardiovascular disease, radiotherapy to the mediastinal/pericardial area and advanced age.

III.5.1.2 Anthracycline-based adjuvant chemotherapy: average benefits

In node-positive breast cancer, anthracycline-based chemotherapy is associated with a 4% absolute risk reduction for recurrence and death above that seen with CMF after 10 years follow-up (11% ($p=0.0005$) and 16% ($p<0.00001$) relative improvements in relapse and death, respectively) (1, 2). In node-negative disease, the absolute advantage of anthracyclines over CMF is smaller (1.7% at 5 years), given the relatively lower baseline risk (1, 2). The benefits achieved by the more active anthracycline schedules must be balanced against increased short and long-term toxicity.

III.5.1.3 Primary chemotherapy (also called neo-adjuvant chemotherapy)

The concept of delivering chemotherapy as primary treatment in early breast cancer patients is attractive because chemosensitivity of the tumor can be assessed "in vivo" allowing for a more "tailored" approach in systemic therapy. This treatment strategy can support further therapy with non-cross resistant agents in cases showing moderate or poor sensitivity to primary chemotherapy. This latter point is particularly attractive because potentially effective “salvage treatments”, such as the taxanes, are nowadays available.

Large international phase II and, more importantly, phase III trials have demonstrated that three to four cycles of primary chemotherapy are feasible and do not compromise either the efficacy of loco-regional treatments (surgery and radiotherapy) or long-term survival (3-6).

Moreover, tumor down-staging achieved after primary chemotherapy can lead to breast-conserving surgery in those patients with large operable tumors, who would otherwise be candidates for mastectomy (3-6).

An additional consideration which strengths the rationale for primary chemotherapy is the proven superiority of chemo-hormonotherapy over hormonotherapy alone in the adjuvant treatment of node-negative ER-positive breast cancer (NSABP B-20 trial) (7). Because of the results of the NSABP B-20 trial, an increasing number of early breast cancer patients is nowadays treated with adjuvant chemotherapy independently of the nodal, menopausal, and hormone receptor status.

The potential advantages of primary medical therapy, and the increasing use of adjuvant chemotherapy in node-negative patients, support the design of a study in which early breast cancer patients will be treated with primary chemotherapy.

III.5.2 The function and pathology of Topoisomerase II alpha

The DNA topoisomerases modulate the topology of DNA by modifying the tertiary structure of the double helix without altering the primary nucleotide sequence. They are responsible for relaxing the torsional stress that accumulates when the DNA double helix unwinds to allow DNA or RNA polymerases access to the genetic code. In the absence of topoisomerases, the accumulation of torsionally strained supercoiled DNA would ultimately interfere with vital cellular functions. During cell division, DNA topoisomerases also function to untangle and physically separate the replicated DNA by facilitating the passage of an intact DNA strand through a double-strand nick in the DNA helix. Thus, two linked circular DNA molecules can be physically separated (decatenated) by the action of specific DNA topoisomerases.

All DNA topoisomerases act by forming temporary single- or double-strand breaks in the double helix in which the enzyme is covalently bound via a tyrosine residue to one of the nicked ends of the phosphodiester DNA backbone. This normally transient intermediate, called the *cleavable complex*, allows for the passage of an intact single or double strand of DNA through this break, resulting in the unwinding or untangling of the DNA molecule. Subsequent religation and release of the enzyme restore the integrity of the DNA double helix.

DNA topoisomerases can be categorized into two broad families, types I and II, based on structure and function. Type I DNA topoisomerases generate transient single-strand breaks in DNA, and these are further divided into subfamilies type IA or IB, depending on whether they form a covalent bond to the 5' or 3' phosphate group, respectively. In contrast, type II DNA topoisomerases generate transient double-strand breaks in DNA, and these are also further subdivided into subfamilies type IIA and type IIB based on differences in protein structure. In higher eukaryotes and humans, three groups of topoisomerases have been identified. One group includes human topoisomerase I and the mitochondrial DNA topoisomerase, which are both type IB enzymes. The second group includes human DNA topoisomerases IIα and IIß, which are type II enzymes, and the final group consists of human topoisomerases IIIα and IIIß , which are both type IA enzymes. The human enzymes with the greatest relevance for cancer chemotherapy are DNA topoisomerase I and DNA topoisomerases IIα and IIß

Human DNA topoisomerase I is a monomeric, 91-kD protein composed of 765 amino acids that is encoded for by the TOP1 gene on chromosome 20. The large human TOP1 gene contains 21 exons extending over 85 kb of DNA. The topoisomerase I protein can be divided into four distinct structural domains.

Human topoisomerase I is uniformly expressed throughout the cell cycle, even in nondividing cells. In mammalian cells, DNA topoisomerase I is essential for cell viability. In comparative studies topoisomerase I protein and messenger RNA expression are higher in malignant tissues, including human ovarian, colon, and prostate cancers, compared to their normal tissue counterparts. This initially raised expectations that topoisomerase interactive agents may selectively target tumors over normal tissues; however, the relative expression of topoisomerase I has not reliably predicted drug sensitivity.

In contrast to topoisomerase I, two homologous but distinct isoforms of type II human topoisomerases have been characterized, DNA topoisomerase IIα and IIß. Human topoisomerase IIα is a 170-kD protein encoded for by a gene on chromosome 17q21-22, whereas the human topoisomerase IIß gene is located on chromosome 3q24 and is associated with a 180-kD protein. Both proteins exist as homodimers, although heterodimerization of IIα and IIß topoisomerases can occur. These homodimers bind to DNA, forming an energy-independent double-strand DNA break in which the proteins are covalently

bound to the 5' end of the broken DNA strands to form the topoisomerase II cleavable complex. In this state, the protein dimer is stabilized by bridging disulfide bonds that literally form a gate in the DNA through which a second intact DNA double-helix strand can pass in an energy-dependent reaction. After strand passage is complete, religation and protein dissociation restore the intact DNA double helix. Topoisomerase I and II can relax positively or negatively supercoiled DNA; however, only topoisomerase II enzymes can decatenate intertwined DNA strands.

In proliferating cells, the expression of topoisomerase IIα varies in different phases of the cell cycle, with maximum expression occurring during the G_2/M phase. In contrast, quiescent cells express low levels of topoisomerase IIα. The ability of topoisomerase IIα to decatenate DNA during cell proliferation suggests that it may be important for the higher-order organization and segregation of newly replicated DNA in chromosomes.[1] Increased topoisomerase IIα activity is associated with transformed malignant cells, and overexpression of topoisomerase IIα is associated with increased tumor aggressiveness in some cancers, such as soft tissue sarcomas. In contrast, topoisomerase IIß expression is relatively constant throughout the cell cycle, suggesting that these two isoforms have distinct but as yet unidentified functions. However, some overlap in activity is present, as overexpression of topoisomerase IIß can rescue proliferating cells that express low levels of topoisomerase IIα.

III.5.2.1 Mechanism of Action of Topoisomerase Interactive Agents

The precise mechanism by which pharmacologic modulation of topoisomerases is converted into cytotoxic drug effects has not been fully characterized. However, the initial interaction between topoisomerase targeting agents and these enzymes is well defined. The majority of topoisomerase interactive agents cause the accumulation of DNA cleavable complexes composed of protein-linked DNA strand breaks. The persistence of these lesions in the presence of ongoing DNA replication or RNA transcription leads to cytotoxic DNA damage, ultimately causing cell-cycle arrest and death by apoptosis or cell necrosis.

III.5.2.2 Topoisomerase II alpha as predictive marker

As we are entering the era of "molecular-based" medicine, it seems to be a high priority to find molecular predictive factors that may enable the clinician to individualize anthracycline-based (neo) adjuvant therapy, i.e. identify those patients most likely to benefit from this class of agents. Topoisomerase II alpha (topo II α) is the molecular target of anthracyclines. In “in-vitro” models, it has been possible to demonstrate a direct correlation between the intranuclear levels of topo II α and the sensitivity to anthracyclines (8). Furthermore, it has been recently reported that topo II α gene amplifications are observed in 5% to 10% of breast cancer patients and that topo II α gene aberrations (either amplification or deletion) are found almost exclusively in HER-2 amplified tumors. This close association between HER-2 and topo II α genes amplification could be explained by the proximity of the two genes on the same arm of chromosome 17: HER-2 gene amplification would be the first genetic event which could lead eventually to topo II α gene aberration (either amplification or deletion) (9). Interestingly, HER-2 amplified breast cancer cell lines have a different degree of sensitivity to anthracyclines according to the topo II α gene status. In cell lines carrying topo II α gene amplification, the sensitivity to anthracyclines is higher than in cell lines carrying a topo II α normal or deleted gene (10). These findings suggest that topo II α could be the main molecular marker predicting the efficacy of anthracyclines, and that HER-2 could act solely as a surrogate predictive marker, mainly because of the concomitant amplification of both genes (11). This hypothesis could explain why some retrospective studies have found that the advantage of an anthracycline-based regimen over a CMF-like treatment in the adjuvant therapy of breast cancer seems to be confined to the subgroup of patients with HER-2 positive tumours (12-17).

III.5.2.3 Clinical evaluation of topo II α as a predictive marker

Preliminary results from a clinical study suggest that complete remission after treatment with anthracyclines for advanced breast cancer is observed only in case of topo II α gene amplification (7 complete remissions, all in patients with topo II α gene amplified tumors, no complete remissions in patients with a normal or deleted topo II α gene) (19).

Moreover, our group has analyzed the predictive value of topo II α in a population of node-positive breast cancer patients randomly treated either with anthracyclines or with CMF (Belgian cooperative trial). In a first study, topo II α was evaluated by immunohistochemistry, which allows the detection of topo II α protein expression. The results of this study suggested that patients deriving the highest benefit from anthracyclines were those in which topo II α protein is immunostained in more than 10% of tumor cells (13). The main findings of this study should be seen as hypothesis-generating because of the limited number of patients evaluated (about fifty in each study arm) and because topo II α protein levels depend on gene amplification as well as on tumor proliferation rate (9). Therefore, topo II α protein expression does not necessarily reflect topo II α gene status (20).

The second study run by our group was based on the same series of patients evaluated in the first study, but, this time, both HER-2 and topo II α genes were evaluated by fluorescence in-situ hybridization (FISH), which allows the detection of gene aberrations (18). The main findings of the second study were quite consistent with the pre-clinical data suggesting that only HER-2 amplified/topo II α amplified tumours show great sensitivity to anthracyclines while the efficacy of these same agents in HER-2 amplified/topo II α non-amplified tumors is comparable to the efficacy of other drugs or regimens like CMF (see disease-free survival curves, figure 1).

Nevertheless, although the results reported in this study bring some additional support to the hypothesis of topo II α as a marker predicting the efficacy of anthracyclines, no definitive conclusions can be drawn because of the fairly limited number of patients evaluated, and the retrospective nature of the analyses.

III.5.3 Anthracyclines and Related Compounds

The anthracycline antibiotics are natural products derived from the actinobacteria *Streptomyces peucetius* var. *caesius*. After its initial isolation, daunorubicin was quickly discovered to induce tumor shrinkage in murine models, and it subsequently demonstrated impressive clinical activity in the treatment of pediatric acute leukaemia. Further research in the 1970s led to the discovery of doxorubicin, a hydroxylated daunorubicin derivative with an extremely broad range of therapeutic activity.

Doxorubicin is commonly used in the treatment of a number of diverse tumor types, including non-Hodgkin's and Hodgkin's lymphoma, multiple myeloma, and lung, ovarian, gastric, thyroid, breast, sarcoma, and pediatric cancers. In contrast, daunorubicin's use is generally limited to the induction treatment of acute leukemia. Several newer anthracyclines have been developed, including epirubicin, a less cardiotoxic doxorubicin analogue with activity in gastric and breast cancer, and idarubicin, a daunorubicin analogue with improved activity as induction therapy for acute myelogenous leukemia (AML). The anthracyclines have the widest range of clinical use of any class of drugs in all of oncology. In spite of their well-defined toxicity profile that includes cardiac toxicity and myelosuppression, they remain relatively easy to combine with other agents and are frequently used in combination chemotherapy regimens.

III.5.3.1 Biochemical and Molecular Pharmacology

Although rapidly recognized as potent anticancer agents, the anthracycline's precise mechanism of action initially remained obscure because of their complex pharmacology. Anthracyclines such as daunorubicin and doxorubicin can directly inhibit cellular helicases, the enzymes that unwind DNA into single strands, and they may also have direct inhibitory effects on topoisomerase IIα independent of cleavable complex stabilization. Thus, some of the anthracyclines may act in part as true topoisomerase enzyme inhibitors. As a consequence of their diverse molecular effects, the ultimate mechanism of cytotoxic action of the anthracyclines may involve multiple different pathways.

Anthracyclines enter cells via passive diffusion, and intracellular accumulation can result in concentrations that are 10- to 500-fold greater than extracellular drug levels. The efficiency of their cellular uptake depends on their lipophilicity, with equilibration occurring more rapidly for daunorubicin than for doxorubicin, and the polar metabolites of both drugs enter cells even more slowly.

- *Clinical Use and Toxicities.* The anthracyclines are most often administered intravenously, typically as bolus or short-term infusions. Epirubicin is most often given as a short, intermittent bolus infusion, whereas daunorubicin and idarubicin are typically administered on a fractionated schedule given daily for 3 or 5 days. Epirubicin is indicated for the treatment of breast cancer, but it also has activity in combination chemotherapy for the treatment of gastric, carcinoid, endometrial, lung, ovarian, esophageal, and prostate cancers and soft tissue sarcomas. Use of daunorubicin and idarubicin is predominantly limited to the induction treatment of adult acute myelogenous and lymphocytic leukemia.

  The major dose-limiting toxicities of doxorubicin are cardiotoxicity and myelosuppression, predominantly neutropenia and leukopenia, with thrombocytopenia and anemia being less severe. Anthracycline-induced myelosuppression is characterized by leukocyte count nadirs occurring 7 to 10 days after drug administration, with recovery occurring by day 21. All anthracyclines can induce cardiac toxicity, which is characterized by acute and chronic effects. Cumulative exposures to anthracyclines are associated with an increased risk of cardiomyopathy and congestive heart failure. Total doses of bolus doxorubicin greater than 400 to 550 mg/m^2 should not be exceeded during a patient's lifetime, especially if the drug is coadministered with other cardiotoxic agents such as radiation therapy or concomitant cyclophosphamide. Epirubicin may have a decreased risk of cardiotoxicity compared to doxorubicin, but serious cardiac dysfunction can occur with any anthracycline. Other common anthracycline-induced side effects include mucositis, alopecia, moderate nausea and vomiting, diarrhea, anorexia, and localized skin reactions, such as pigmentation changes, local irritation, radiation sensitization, and inflammation at sites of prior radiation therapy (radiation recall). Prophylactic antiemetics are routinely given with bolus doses of doxorubicin, and all patients should be cautioned to expect their urine color to redden after drug administration. Prolonged infusions may reduce the risk of cardiotoxicity and decrease nausea and vomiting, but they may also increase the risk of mucositis and extravasation. Anthracycline infusions should be administered carefully, with close observation of all infusion sites.
- *Clinical Pharmacology.* Short intravenous infusions of doxorubicin are associated with a triphasic clearance profile of plasma elimination with a large volume of distribution of approximately 800 L/m^2. Distribution occurs rapidly as the drug concentrates in cells and tissues, with an initial distribution half-life of 5 to 10 minutes, a secondary half-life of 1 to 3 hours, and a prolonged terminal elimination half-life of 24 to 50 hours. The measured half-lives of epirubicin are similar; however, the total clearance of this analogue is approximately twofold higher than for doxorubicin, consistent with its greater tissue penetration and increased metabolism.

Anthracycline drug clearance is predominately mediated by hepatic metabolism and biliary excretion. A common metabolic pathway is the reduction of the anthracycline ketone group by aldoketoreductases to form polar alcoholic metabolites, such as doxorubicinol and daunorubicinol. Typically, the biologic activity of these 13(S)-dihydro-derivatives is slightly less than the parental compounds because of reduced lipophilicity and decreased cellular penetration. However, idarubicinol is an exception. Its biologic activity is similar to that of idarubicin, which may contribute to its greater efficacy over daunorubicin in the treatment of acute leukemia. Aldoketoreductases are widely distributed throughout the body, with high activity found in the liver and in erythrocytes. Daunorubicin and idarubicin are avid substrates for this enzyme, rapidly forming daunorubicinol and idarubicinol, both of which have extended half-lives and circulate in plasma at concentrations that exceed those of the parental compounds. In contrast, doxorubicin and epirubicin are less avidly metabolized by this route, and as a consequence, their metabolites, doxorubicinol and epirubicinol, are much less important. Anthracyclines can also undergo enzymatic deglycosylation to form inactive aglycones, which can contribute to overall drug clearance. Epirubicin has a unique steric orientation of the C-4 hydroxyl group, making it the only anthracycline substrate for conjugation reactions mediated by glucuronyltransferases and sulfatases.

All anthracyclines should be dose reduced in patients with hepatic dysfunction. Historically, dose adjustments have been recommended based on the degree of hyperbilirubinemia. Caution is warranted when doxorubicin is coadministered with paclitaxel. The mechanism underlying this pharmacokinetic interaction is not known. Another potentially serious drug interaction is enhanced cardiotoxicity of trastuzumab, when coadministered with doxorubicin. Trastuzumab by itself is associated with left ventricular dysfunction and congestive heart failure, and these risks are enhanced when it is combined with anthracycline therapy. Concomitant use of these potentially cardiotoxic agents is generally contraindicated.

- *Anthracycline Cardiotoxicity.* Anthracycline-induced cardiotoxicity may be either acute or chronic. Acute effects include electrocardiographic changes such as sinus tachycardia, ectopic contractions, nonspecific ST and T-wave changes, decreased QRS voltage, prolonged QT intervals, and heart block. These acute toxicities are generally reversible and clinically insignificant, and they do not predict future cumulative drug-related cardiac complications. A potentially more severe acute pericarditis-myocarditis syndrome can also occur within 1 or 2 days after anthracycline administration. In contrast, chronic anthracycline-induced cardiotoxicity is characterized by myocardial dysfunction and congestive heart failure, most often starting after 1 year of treatment. It is typically irreversible and is associated with cumulative drug exposure. However, the risk of chronic cardiotoxicity may vary, and it is heavily influenced by other factors, including a history of chest irradiation or coadministration of additional agents, such as paclitaxel, cyclophosphamide, or trastuzumab. Other potential risk factors include female gender, treatment at a young age, and any prior or concomitant heart disease.

III.5.4 The present study protocol

Supported by "in-vitro" and preliminary "in-vivo" data, briefly summarized above, this study is designed to test prospectively the value of topo II alpha gene amplification and protein overexpression in predicting the efficacy of anthracyclines. To our knowledge this is the only prospective trial worldwide which is attempting to prospectively clarify the predictive value of this interesting biological marker. This study could have important practical implications in the daily clinical management of early breast cancer patients because, if the trial confirms that topo II α gene amplification and/or protein overexpression are associated with high efficacy of anthracyclines, while topo II α normal/deleted gene and low protein content are associated

with modest efficacy, an important step forward in the direction of anthracycline "tailoring" would be accomplished.

The practical advantage of this approach would be to use anthracyclines primarily in patients who are supposed to derive the largest benefit, thus sparing the long-term anthracycline-related toxicity (i.e. secondary acute myeloid leukemia, cardiac dysfunction, and amenorrhea/sterility in case of fertile women) to those patients for whom no significant gain in antitumor activity is anticipated.

To reach this ambitious aim, early breast cancer patients with tumors of at least 2 cm (defined by breast ultrasound) will be evaluated for topo II α gene and protein expression. For this purpose, a pre-treatment biopsy (tru-cut) will be performed and topo II α gene will be evaluated on fixed samples by FISH. The use of a triple probe will allow the concomitant evaluation of the HER-2 gene status. Topo II α protein will be evaluated by immunohistochemistry (IHC). Afterwards, all patients, independently of the topo II α gene and protein status, will be treated with single-agent epirubicin (see study design, chapter n° 2, and study registration and logistics, chapter n°15).

Eligibility criteria will allow the participation of patients for whom the use of an anthracycline-based adjuvant therapy would have been most probably proposed after breast cancer surgery, mainly because of estrogen receptor (ER) negativity (see chapter n°4 for eligibility criteria). Therefore, no overtreatment with anthracyclines will occur in this group of patients. Pathological complete response (pCR) to epirubicin will be correlated with the topo II α gene and protein status. The study has two biological hypotheses, one for the subgroup of patients with ER negative/HER-2 amplified tumors, the other one for the subgroup of patients with ER negative/HER-2 non amplified tumors.

- **1st hypothesis**: Patients with ER negative/HER-2 amplified tumors:
  In this subgroup of patients, topo II α gene will be amplified in about 40% of cases. We hypothesize that in topo II α amplified tumors a three-fold increase in pCR rate will be observed, as opposed to the pCR rate in tumors with topo II α normal or deleted gene.
- **2nd hypothesis :** Patients with ER negative/HER-2 non amplified tumors:
  In this subset of patients, almost no topo II α gene aberrations will be found based on previous data discussed above. However, recent data reported by C. Sotiriou *et al* using cDNA microarrays, suggest that in this subset of ER-negative HER-2 negative tumors, also defined as the basal-like subset, two distinct subgroups can be identified (i.e. basal-like 1 and 2). While basal-like 1 tumors show a high proliferation rate and high levels of topo II α RNA, basal-like 2 tumors have a moderate-low proliferation rate and normal levels of topo II α RNA (20). We hypothesize that the topo II α RNA overexpression in basal-like 1 tumors is not related to topo II α gene amplification because no concomitant HER-2 gene amplification is reported in this subset of tumors. The second study hypothesis is that in ER negative/HER-2 non amplified tumors with topo II α protein overexpression, a 2.5 fold increase in pCR rate will be observed, as opposed to the pCR rate in tumors with low topo II α protein content.

  A tumor sample drawn at the time of pre-treatment biopsy will be frozen and used to perform oligonucleotide based microarrays (Affymetrix). This technique allows the evaluation of thousands of genes and ultimately provides us with the tumor genetic profile (22). Homogeneous genetic profiles (genetic clusters) that might be identified, will be correlated with the efficacy of single-agent epirubicin. This correlation will allow us to address the secondary end-point of this study, which is the identification of other genes or eventually a genetic profile playing a role in the determination of sensitivity to anthracyclines. Among the genes that could interfere with sensitivity to anthracyclines, p-53 seems to deserve special attention. Indeed, "in-vitro" data suggest that at least some p-53 mutated tumors are poorly sensitive to anthracyclines, primarily because

anthracycline-induced apoptosis is prevented (23). Interestingly, p-53 mutated tumors display frequently HER-2 gene amplification and therefore topo II α gene amplification (24). Accordingly, p-53 mutations could hamper response to anthracyclines even in tumors carrying topo II α gene amplification. This hypothesis will also be explored in the present study, because p-53 mutations will be evaluated by DNA sequencing, and the efficacy of epirubicin in topo II α amplified and non-amplified tumors will be correlated with p-53 status.

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III.6 Rationale for inflammatory and locally advanced breast cancer

III.6.1 Epidemiology and characteristics of the disease:

In a recent survey from Surveillance, Epidemiology, and End Results (SEER) female breast cancer records, 1.3 % women with inflammatory breast carcinoma (IBC) and 4.6 % with locally advanced breast carcinoma (LABC) were identified (1).

Inflammatory breast cancer, perhaps the most aggressive form of breast neoplasia represents 1 to 3% of newly diagnosed breast malignancies. African Americans have a higher incidence of IBC than do Caucasians and other ethnic groups. The entity is diagnosed on clinical grounds, based on the presence of erythema and edema (peau d'orange) of the skin of the breast, as well as ridging (2). Most inflammatory cancers present as diffuse infiltration of the breast without a well-defined tumor. Dermal lymphatic invasion is present in most patients, but this feature is not a necessary component of the diagnosis. Most IBC are poorly differentiated ductal carcinomas and are ER- and PR- negative. HER2 overexpression and p53 gene mutations are also frequently found abnormalities in IBC. Locally advanced breast cancer encompasses a heterogeneous group of patients including those with neglected slow growing tumors as well as those with biologically aggressive disease. LABC is a relatively uncommon presentation in the economically developed world accounting for 5 % of cases in major centers. However, in medically underserved area and in many countries, LABC represents 30 to 50% of newly diagnosed breast cancers.

III.6.1.1 Benefits of neo-adjuvant chemotherapy in inflammatory and locally advanced breast cancer

Inflammatory and locally advanced breast carcinomas are both associated with poor prognosis.

Before the introduction of systemic therapy, IBC was a uniformly fatal disease. The local recurrence rate was very high (50% to 80%) and metastases developed in more than 90 % of patients in less than 2 years. Nowadays, objective response rates after induction chemotherapy can reach 80%. Three year survival rates, after combined modality therapy, range from 40% to 70% and at 5 years, up to 50 % of patients remain alive (3, 4).

With surgery and/or radiotherapy alone, the prognosis in LABC is very poor. This poor long-term outcome prompted the introduction of primary chemotherapy or hormonotherapy, with the first reports published in the 70's (5). Such a multimodality approach has led to a significant improvement in LABC outcome. Clinical complete remissions were reported in 10 to 20% of patients treated in this manner in most clinical trials. However, only two thirds of the patients with a clinical complete response are found to have a pathologic complete response (pCR). Several authors have demonstrated that the achievement of a pCR is an excellent predictor of long-term survival. With standard anthracycline-based regimens, pCR rates range from 3.5% to 12% (6, 7). The addition of taxanes to anthracyclines in the neo-adjuvant regimen significantly increases pCR and improves survival of patients who achieve pCR (8, 9).

III.6.1.2 Dose-dense chemotherapy:

Dose intensity (expressed in mg/m^2/w) can be increased either by dose escalation and/or by reducing the interval between the cycles. Interest in dose-intensity is based on the observation that, in experimental models, a given dose kills a certain fraction rather than a certain number of exponentially growing cancer cells. The initial *in vitro* mathematical model of gompertzian curve (exponential growth and log cell kill by cytotoxic agent) has been adapted and extended to human cancer and particularly breast cancer to explain kinetic heterogeneity. (10, 11, 12) In order to maximize tumor cell killing and to circumvent emerging drug resistance, both ways of increasing dose intensity have been tested.

Results of dose intensification by increasing dose of chemotherapy (high dose chemotherapy with stem-cell support) have been disappointing in the adjuvant setting (13, 14, 15).

More recently, the dose density hypothesis, which refers to the administration of drugs with a shortened inter-treatment interval, has been tested. It has been hypothesized that a more frequent administration of cytotoxic therapy could be a more effective way of minimizing residual tumor burden than dose escalation. Hematopoietic growth factors have made it possible to test dose-dense chemotherapy since they allow faster neutrophil recovery and delivery of chemotherapy on time. In a prospective and well designed adjuvant trial, 2005 women with node positive breast cancer were randomized between conventional or dose-dense chemotherapy. (16) In the dose-dense arm, the same chemotherapy was administered every two weeks with hematopoietic growth factors support, instead of every three weeks in the conventional arm. Chemotherapy consisted of adriamycin 60 mg/m2 (A), paclitaxel 175 mg/m2 (T) and cyclophosphamide 600mg/m2 (C) administered either in combination (AC$\rightarrow$T) or sequentially (A$\rightarrow$T$\rightarrow$C). Dose-dense treatment improved both disease-free survival and overall survival and, interestingly, was not more toxic. Indeed, the use of granulocyte-colony stimulating factor in the dose-dense regimen resulted in a statistically significant decrease in neutropenia.

III.6.1.3 Clinical studies addressing dose dense chemotherapy in the neoadjuvant setting

In LABC, a relatively small trial (n = 448), run by EORTC/NCIC/SAKK (17), compared a conventionally dosed neo-adjuvant regimen (6 courses of Canadian CEF with 60 mg/m2 epirubicin, every 28 days) to a dose-dense regimen (6 courses of EC with 120 mg/m2 epirubicin, every 14 days, with granulocyte-colony stimulating factor support). The study failed to show an improvement in disease free survival (DFS) with the dose dense combination. However, it is interesting to note that, with a median follow-up of 5 years, the short dose-dense regimen was as effective as the longer CEF treatment, with no increased rate of cardiotoxicity or leukemia.

In a small Italian study (n = 150), LABC patients were randomized to neo-adjuvant and adjuvant chemotherapy every three weeks or every two weeks (18). Neo-adjuvant chemotherapy consisted of three courses of FEC (with Epirubicin 60 mg/m^2) and adjuvant chemotherapy consisted of three courses of FEC alternated with three courses of CMF. No difference in the pathological response rate was observed (the primary endpoint).

These two negative trials in LABC do not mean that dose-dense chemotherapy is of no benefit in LABC. Firstly, these two trials were probably too small to show a significant difference. Secondly, there might be a benefit for a subgroup of patients with LABC, as suggested by the unplanned subset analysis of the Baldini trial. Thirdly, in these two trials, there was no prospective stratification according to estrogen-receptor status.

In the present study, we plan to use a dose-dense administration of epirubicin (100 mg/m^2/2 weeks). We keep the same drug as for early breast cancer but we use a slightly more aggressive regimen with a higher dose-density. The feasibility of the administration of epirubicin 100 mg/m^2 every two weeks with granulocyte-growth factor support has been shown in the neoadjuvant, metastatic (19) and adjuvant settings (20) with acceptable toxicity. This neoadjuvant epirubicin regimen may be completed by adjuvant chemotherapy, such as taxane-based regimens, since the sequential approach (anthracyclines $\rightarrow$ taxanes) has been suggested superior to anthracyclines regimen in LABC (21).

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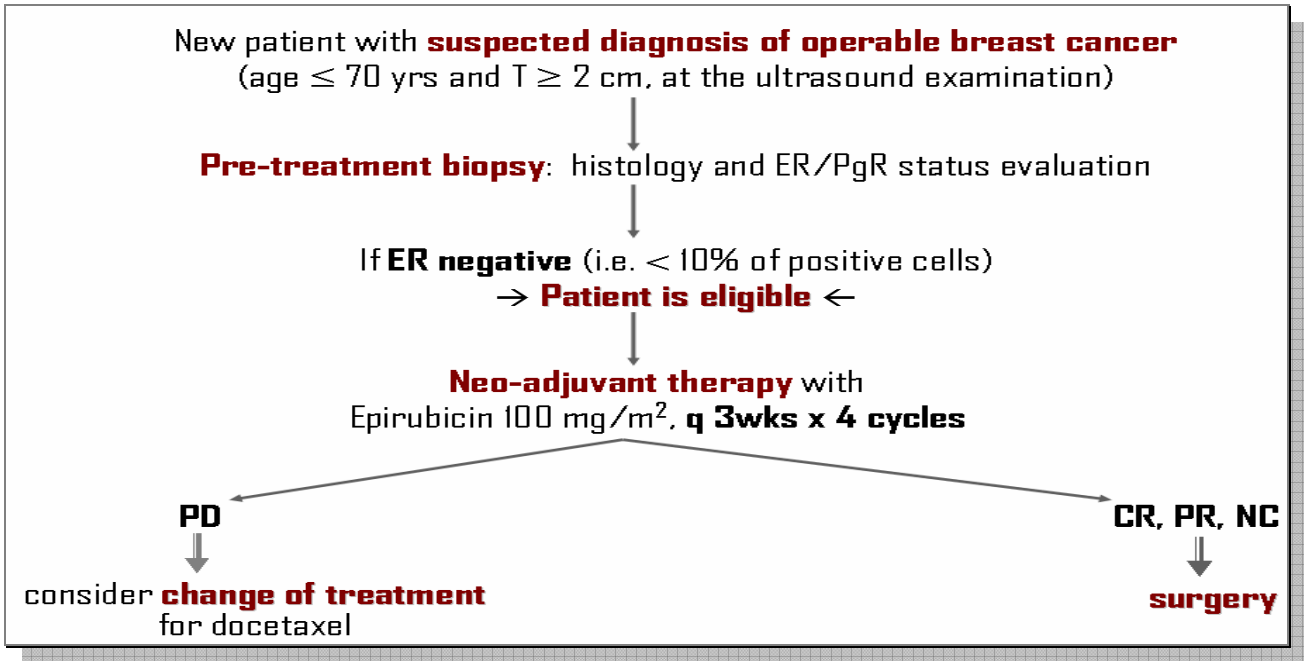
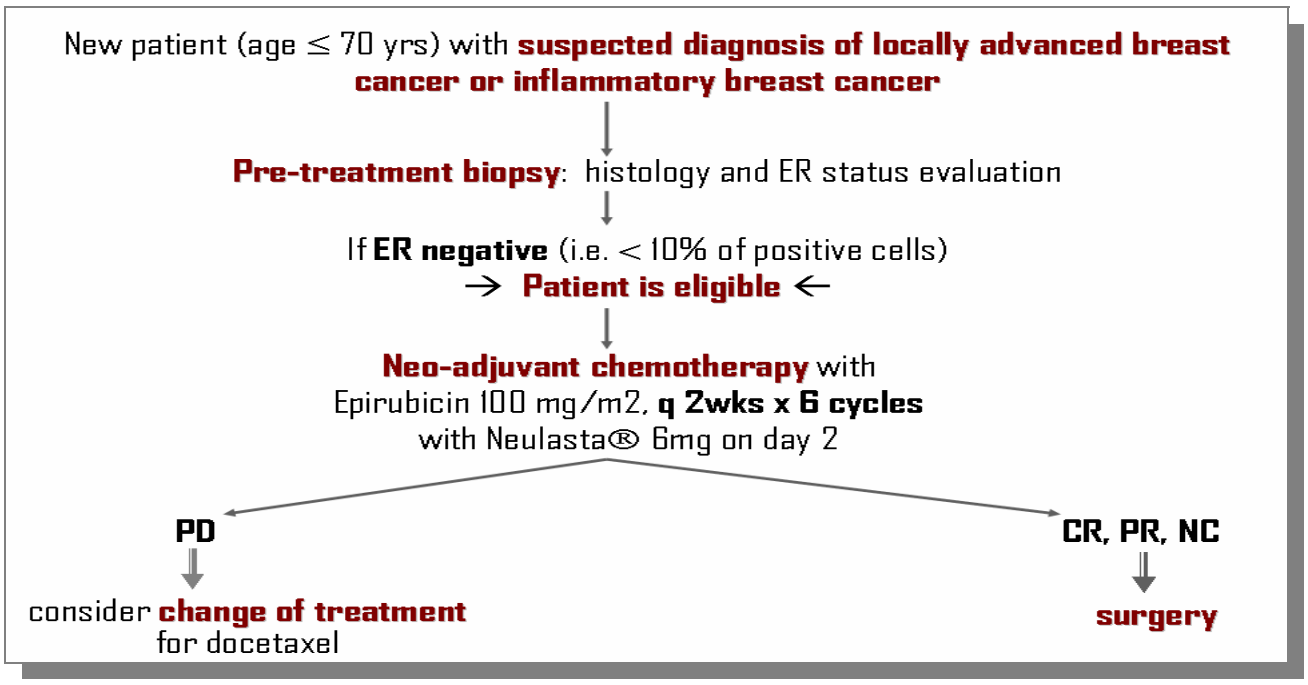
III.7 Study Design

A. Early Breast Cancer



After surgery, adjuvant chemotherapy should be considered, preferably with docetaxel however adjuvant treatment is not part of this protocol. Participation to the HERA protocol or to an adjuvant hormonal therapy protocol is allowed.

B. Locally Advanced And Inflammatory Breast Cancer

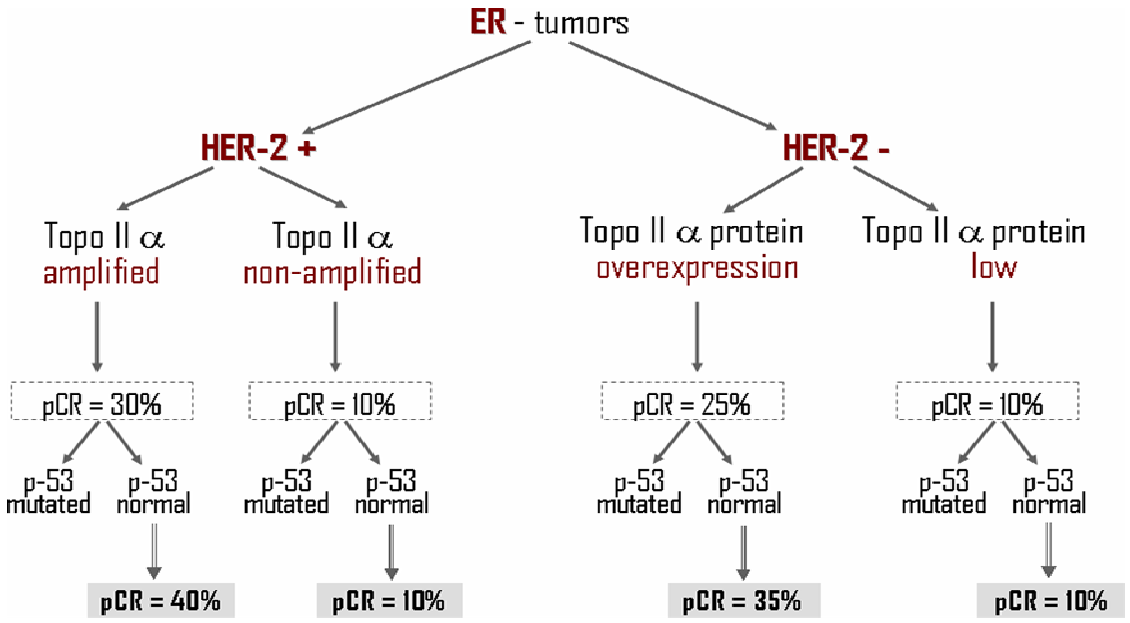


III.8 Study Hypothesis

Primary	1)	ER negative/HER-2 amplified tumors: in case of topo II α gene amplification, the pathologic complete remission (p CR) rate is expected to be 30%, while in absence of topo II α gene amplification the p CR rate is expected to be 10%.
	2)	ER negative/HER-2 non amplified tumors: in case of topo II α protein overexpression, the pCR rate is expected to be 25%, while in absence of topo II α protein overexpression, the pCR rate is expected to be 10%.
Secondary	3)	ER negative/HER-2 amplified tumors: in case of topo II α gene amplification, the pathologic complete remission (p CR) rate is expected to be 30%, while in absence of topo II α gene amplification the p CR rate is expected to be 10 %.
	4)	ER negative/HER-2 non amplified tumors: in case of topo II α protein overexpression, the pCR rate is expected to be 25%, while in absence of topo II α protein overexpression, the pCR rate is expected to be 10%.

Exploratory analyses
1) If topo II α protein overexpression will have a predictive value (see primary study hypothesis, point b), the following comparison will be done in the subgroup of patients with ER negative/HER-2 amplified tumors: pCR rate in the subgroup of patients with topo II α gene amplification and high topo II α protein content vs pCR rate in the subgroup of patients without topo II α gene amplification and low topo II α protein content.
2) The following comparison will be done independently of the HER-2 status: pCR rate in the subgroup of patients with topo II α gene amplification or topo II α gene non amplified and protein overexpression vs pCR rate in the subgroup of patients without topo II α gene amplification and low topo II α protein content.
3) The tumor genetic profile will be identified through oligonucleotides microarrays and correlated with the efficacy of epirubicin, in an attempt to identify a pattern of genes involved in the response to this drug (predictive gene signature).
4) Disease-free (DFS) and overall survival (OS) analyses will be recorded for all patients entered in the trial.
5) Efficacy of neo-adjuvant epirubicin will also be correlated with the HER-2 gene status.

⇨ **Summary of the study hypotheses**



III.8.1 Eligibility Criteria

Inclusion Criteria	
1)	Histologically-confirmed breast cancer (either operable, or locally advanced or inflammatory)
2)	Age ≤ 70 yrs
3)	Female patient
4)	Tumor size ≥ 2 cm at the ultrasound examination.
5)	ER-negative tumors, defined according to immunohistochemistry (i.e. < 10% of positive cells after immunostaining).
6)	Multifocal and multicentric breast tumors are allowed if all foci are ER-. It is reasonable to limit multifoci tumors to bifocal ones since a fixed and frozen samples should be obtained from each focus.
7)	Fixed and frozen samples from the primary tumor, obtained before treatment with epirubicin, must be available for evaluation of biological markers (topo II α gene and protein, HER-2 gene, p-53 gene, oligonucleotides microarrays).
8)	Written informed consent before study registration.
9)	Performance status ≤ 1 (ECOG scale)
10)	ANC ≥ 1500/mm3, platelets ≥ 100.000/mm^3, Hb ≥ 10 g/dl, total bilirubin and serum creatinine ≤ 1 N, GOT/GPT ≤ 1.5 N, alkaline phospatase ≤ 2.5 N
11)	Normal left ventricular ejection fraction by echocardiography or muga scan
12)	Negative pregnancy test for all women of childbearing potential. Patients of childbearing potential must implement adequate non-hormonal measures to avoid pregnancy during treatment.

Exclusion Criteria	
1)	Metastatic breast cancer
2)	Serious medical conditions like: ▪ congestive heart failure or unstable angina pectoris, previous history of myocardial infarction within 1 year from study entry, uncontrolled arrhythmias; ▪ history of significant neurologic or psychiatric disorders; ▪ active uncontrolled infection; ▪ active peptic ulcer, unstable diabetes mellitus.
3)	Concomitant contro-lateral invasive breast cancer
4)	Concurrent treatment with hormonal replacement therapy
5)	Concurrent treatment with any other anti-cancer therapy
6)	Previous treatment with anthracyclines for breast cancer.

III.8.2 Pre-treatment staging and examinations during chemotherapy

1) Pre-treatment staging will include:
   - physical examination ; measurements of the breast tumor must be indicated;
   - hemato-chemistry survey (hematology, GOT/GPT, alkaline phosphatase, total biluribin, creatinine)
   - blood sample for research purposes (see paragraph 9.4);
   - EKG and LVEF assessment by muga scan or echocardiography;
   - chest-x-ray, bone scan (confirmatory x-ray or CT-scan in case of hot spots), liver ultrasound;
   - bilateral mammography and breast ultrasound. Measurements of the breast tumor must be indicated.

   Pre-treatment investigations should be done within 4 weeks of the beginning of the treatment.

2) Examinations required during chemotherapy:
   - hemato-chemistry survey (red blood cells, Hb, platelets, white blood cells, absolute neutrophil count, total bilirubin, serum creatinine, GOT/GPT, alkaline phosphatase) to be repeated every 21 days, before each cycle of chemotherapy;
   - clinical measurements of the breast tumor before each chemotherapy course.

3) Examinations required after the fourth cycle of epirubicin in case of early breast cancer and after the sixth cycle in case of LABC or IBC : physical examination, mammography and breast ultrasound. The latter will be used to evaluate the radiological response to epirubicin

III.8.3 Epirubicin administration

Epirubicin will be administered at the dose of 100mg/sqm day 1 I.V. once every three weeks for four consecutive cycles for early breast cancer and once every two weeks with pegfilgrastim (Neulasta®) support (6mg, one single sc administration on day 2) for locally advanced and inflammatory breast cancer. The first cycle will start within three weeks from the date of patient's registration into the study.

- ***Epirubicine dose-modification or delays according to side effects***
  Treatment delay. Delay will be allowed in case of no bone marrow recovery at day 14 for dose- dense therapy and at day 21 for conventional schedule (i.e. ANC $<$ 1500 and/or platelets $<$ 100.000). Hemogram will be repeated every 3-4 days until recovery will occur. The use of G-CSF to accelerate recovery with the q 3week regimen will be left at the discretion of the investigator. In case of neutropenic fever (i.e. T $>$ 38° and concomitant ANC $<$ 500/mm³), the next cycle will be delayed until full recovery. Dose-reductions because of one previous episode of neutropenic fever should be avoided. The use of prophylactic antibiotics (and/or G-CSF for the q 3 week regimen) for the secondary prevention of neutropenic fever are strongly recommended. Delay will be allowed also in case no full recovery from non-hematological toxicity has occurred by day 14 for dose-dense therapy and by day 21 for conventional schedule: in case of liver and/or kidney chemistry abnormalities (i.e. GOT and GPT $>$ 2 N, or alkaline phosphatase $>$ 2.5 N, or total bilirubin $>$ 1.5 N, or serum creatinine $>$ 1.5 N), treatment will be delayed until recovery.

- ***Treatment dose-reduction***
  A 25% reduction in the dose of epirubicin will be mandatory in case one of the following side-effects is observed:
  a) vomiting grade 4 despite adequate anti-emetic therapy;
  b) stomatitis and/or diarrhea $\geq$ grade 3;
  c) two episodes of neutropenic fever;
  d) one episode of severe infection.

- ***Treatment withdrawal***
  Treatment withdrawal will be mandatory in case of:
  a) clinical and/or instrumental evidence of congestive heart failure or of any other severe cardiac disease;
  b) persisting toxicity despite a maximum of two weeks of delay and/or dose-reductions have been implemented according to the guidelines reported in this chapter.

  In case the treatment will be prematurely discontinued, the patient will not be evaluable for the study and will be replaced by a new patient.

- ***Clinical response evaluation.*** Clinical-radiological response evaluation will be done according to the standard RECIST criteria (Therasse P et al, J Natl Cancer Inst 92:205-16, 2000). Breast ultrasound will be used to evaluate the tumor response.

- ***Procedures for tumor biopsy, tissue samples handling and biological markers evaluation***
  - *Tumor biopsy.* Tumor core biopsy (no fine needle aspiration, no incisional or excisional biopsy) will be performed in case the patient is potentially eligible (i.e. age $\leq$ 70 years and tumor diameter $\geq$ 2 cm with breast ultrasound).
  - *Tissue samples handling.* At diagnosis, two samples will be fixed in formalin, one for routine analysis, the second for research purpose. Two other samples will be frozen within 5 minutes, according to the following procedures:

- ✓ put the biopsy in the tissue tek recipient;
- ✓ add OCT by Sakura on biopsy (OCT: compound to bind tissue to the specimen block and to surround and cover the tissue specimen);
- ✓ put tissue tek recipient with biopsy in liquid nitrogen;
- ✓ transfer tissue tek recipients to –80°C freezer.

Both fixed and frozen samples will be used to perform biological markers evaluation.

- ✓ A second (optional) biopsy will be performed after the first epirubicin cycle (on day 14 or 21) for consenting women. The same samples (in formalin and frozen) should be taken as at diagnosis.
- ✓ At time of surgery, two frozen and one formalin samples will be collected.

- ***Serum, whole blood and urine samples***

A. Serum. Serum will be collected for post genimic analyses at 5 different times :
1) at diagnosis;
2) after the first epirubicin chemotherapy cycle;
3) at surgery time;
4) just before adjuvant chemotherapy;
5) at the end of adjuvant chemotherapy. For logistic reasons, this last serum sample will be collected just before the last chemotherapy cycle instead of at the end of chemotherapy.

B. Whole blood sample. A whole blood sample will be collected before starting treatment from each consenting patient and stored at –80°C. DNA will be extracted from the PBMC by conventional methodology and analyzed for polymorphisms in candidate genes that may reveal the likelihood of benefit or side-effects from the chemotherapy. This will involve germ-line DNA analysis. Data from this analysis will not be provided to the patient or her physician. Venus blood (3 x 10ml) will also be collected in plain plastic tubes at each of the above intervals from patient and healthy individual/control participating in the matabolomic study.

C. Plasma collection and storage. 5ml out of the 10ml from each collected blood sample will be incubated in room temperature (RT) for 2-3 hours and then centrifuged in RT under 3600g for 5 min. Subsequently, the supernatant (S1) of the centrifuged samples will be collected and re-centrifuged under the same conditions as in the first centrifugation, followed by subsequent collection of the supernatant (S2) which will be weighted and stored at -80^{o}C. At this point, the plasma samples could be mailed in dry ice to the metabolomics laboratory for further analysis.

D. Lymphocytes isolation. Lymphocytes isolation from the remaining 5ml of each blood sample will be carried out at the laboratory that collects the blood samples based on the standard protocol for lymphocytes isolation. The lymphocytes will be frozen at -80^{o}C. At this point, the samples will be mailed in dry ice to the metabolomics laboratory for further analysis.

E. Urine collection and storage. Urine samples (3x10 ml) will be collected every patients participating the metabolomic study and healthy individual/control participating in the study, weighted and stored at -80^{o}C. At this point, the samples may be sent by mail in dry ice to the metabolomics laboratory for further analysis.

III.8.4 Biological markers evaluation

HER-2 and topo II α genes will be evaluated by fluorescent in-situ hybridization (FISH) with the Multicolor topo II α spectrum orange, HER-2 spectrum green and CEP17 spectrum aqua probe by Vysis (Illinois, USA). The tumor will be classified as topo II α gene amplified if the

ratio between the topo II α gene copy number and the centromere 17 copy number will be $\geq$ 1.5. (18).

The tumor will be classified HER-2 amplified if the ratio between the HER-2 gene copy number and the centromere 17 copy number will be $\geq$ 2. (18).

Topo II α protein levels will be evaluated by IHC with Boerhinger Manheim antibody clone KiS1 (13).

Estrogen and progesterone receptors (ER and PgR) will be evaluated locally by IHC. ER and PgR scores will be expressed as percentage of tumor cells with positive staining. Tumors will be defined as ER-negative if $<$ 10% of tumor cells will have positive immunostaining. PgR score will not be used to decide whether the patient is eligible for the study (see eligibility criteria, chapter 4.0). RNAs from frozen samples will be analyzed using oligonucleotides microarray (Affymetrix). p-53 gene mutations will be evaluated by DHPLC , and confirmed by DNA sequencing of the mutated exons.

Other biological markers, like surviving for exemple, might be analyzed according to scientific progress. ER and PgR status will be confirmed centrally at the Jules Bordet Institute with Novocastra antibodies (clone 6F11 for ER; AB for PgR) using an automated immunostainer (Nexes, Ventana).

- ***Gene expression profiling.*** U133 Plus 2.0 GeneChip® (Affymetrix®) arrays containing approximately 47,000 genes will be used. For all the samples an H&E (hematoxylin-eosin) stained section will be prepared prior to cutting slides for RNA isolation to assess tumor cell percentage; only samples with >70% tumor cells will be considered.

- ***Genotyping.*** Genotyping will be done using the Affymetrix 500K arrays, according to protocols recommended by Affymetrix. The GeneChip Human Mapping 500K Array Set enables highly powered whole-genome association studies across different populations. It is comprised of two arrays which enable genotyping more than 500,000 SNPs enabling truly high-powered, whole-genome association studies.

- ***Comparative Genomic Hybridization (CGH).*** Array-based CGH will also be done using either the 100K or the 500K Affymetrix arrays, according to protocols recommended by Affymetrix.

- ***Metabolic profiling.*** The samples will be received by the metabolomics laboratory and will be analyzed based on the protocol optimized from that described in Kanani and Klapa, 2006. The various steps that will be followed are described below:
  1. **Extraction.** The dried polar metabolite extracts of the samples will be obtained following a protocol based on those described in [Roessner et.al., 2000; Kanani and Klapa, 2006]. Part of the samples from healthy individuals will be initially used to standardized the protocol in the context of the particular fluids.
  2. **Derivatization.** Each dried metabolite extract will be transformed into its TMS-derivative mixture in two steps. First, it will react with methoxyamine hydrochloride solution in pyridine. Subsequently, the samples will be supplemented N-methyl-trimethylsilyl-trifluoroacetamide (MSTFA), and allowed to react, according to the derivatization strategy presented in [Kanani and Klapa, 2006].
  3. **GC-MS runs.** Each of the derivatized samples will be run at least three times (≥3 injections) through the Saturn 2200 GC-(ion trap) MS (Varian Inc.) as described in [Kanani and Klapa, 2006]. Peak identification will be based on mass spectra and retention times from (i) own library of standards, (ii) the publicly available Max-Planck based curated TMS-derivative library [http://www.mpimp-golm.mpg.de/mms-library/index-e.html], and (iii) the commercially available NIST MS-library [Ausloos et al., 1999]. The peak areas of all identified peaks after the normalization step described in Kanani and Klapa, 2006 will be exported in Excel spreadsheet.

III.8.4.1 Data Analysis

The acquired profiles will be analyzed using multivariate statistical analysis (of time-series nature in particular for some of the cases). Specifically, metabolomic profiling analysis requires the same hypothesis testing (SAM, t-test, ANOVA, etc) as well as clustering analysis algorithms to be existing in the shared Grid.

In addition to these techniques, it is required that the metabolomic analysis results can be depicted in the context of the metabolic network, preferably in conjunction with –omic data of other type (in this case, transcriptomic), which are acquired for the same samples. For this, a database which connects gene annotation, to the enzyme name, to the reaction that this catalyzes, to the stoichiometry of this reaction (it would be better if we could get use the maps of KEGG database, www.kegg.com, or get in touch with Ecocyc to obtain their database – which is the most robust at this point -). Having such a database, we would need a tool that could "read" the data from the Excel spreadsheet (or after the multivariate statistical analysis) and based on a color-code populate the various metabolic maps. In this way, it will be easy for the researcher to see which metabolic pathways are significantly affected by the particular disease and/or therapeutic regimen and to what extent, being able to derive biologically relevant conclusions.

III.8.5 Breast cancer Surgery

Surgery will be performed three to five weeks after the fourth (for early BC) or sixth (for LABC and IBC) cycle of neo-adjuvant therapy with epirubicin.

Breast-conserving surgery or mastectomy will be performed at the discretion of the participating surgeon.

Concomitant ipsilateral axillary dissection will be performed. No exclusive sentinel node biopsy will be allowed in this study because neo-adjuvant therapy could interfere with the level of reliability of this surgical procedure. However, sentinel node biopsy will be allowed if followed by standard axillary dissection.

The choice of labeling the tumor before starting neo-adjuvant chemotherapy will be left at the discretion of the participating surgeon/radiologist.

III.8.5.1 Pathologic response

At the time of pathology examination, the following scenarios will be possible:

a) persistence of macroscopic invasive breast cancer in the breast and/or in the axillary nodes;
b) persistence of microscopic invasive breast cancer in the breast and/or in the axillary nodes;
c) pathological complete response (pCR): absence of residual invasive breast carcinoma (macro and microscopic) in the breast and in the axillary nodes. Persistence of in-situ carcinoma will not interfere with the definition of pCR.

The definition of pCR will require the examination of a minimum of ten sections from the original site of the primary tumor and will be guided also by the gross examination. Moreover, all axillary lymph nodes, smaller than 1 cm, will have to be entirely examined through 2 mm sections. In case of lymph nodes of at least 1cm, two sections of the macroscopically most suspicious areas will have to be examined. A sample of the remaining tumor must be collected for research purposes.

III.8.6 Post-operative treatments

- ***Radiotherapy.*** It is mandatory to administer radiotherapy in case of breast-conserving surgery. Radiotherapy after mastectomy will be administered at the discretion of the investigator. Radiotherapy will be performed either during or after adjuvant chemotherapy,

if any, or after breast cancer surgery if no adjuvant chemotherapy will be administered. In case of adjuvant hormonal therapy, concomitant radiotherapy will be allowed.

- ***Hormonal therapy.*** Adjuvant hormonotherapy is recommended in all patients with potentially endocrine-responsive tumors (in this trial, patients with PgR positive tumors). The decision whether to administer or not adjuvant hormonotherapy will be left at the discretion of the participating investigator.
- ***Adjuvant chemotherapy.*** Adjuvant chemotherapy outside or in the context of a clinical trial is allowed. The use of docetaxel is recommended athough it is not part of this protocol.
- ***Participation in adjuvant therapy trials.*** Patients treated in the present neo-adjuvant trial will be allowed to participate in any adjuvant trial evaluating chemotherapeutic, hormonal or biological agents.

⇨ **Follow-up.** Schedule of follow-up includes physical examination performed every 4 months for the first two years and thereafter every 6 months for the next three years. After 5 years, an annual follow-up until death is preferable. Bilateral mammography is recommended annually.

III.8.7 Statistics

The patient population will be stratified according to HER-2 status and p-53 status:

1. In **HER-2 positive** patients, the two hypotheses of interest are:

   a) that there is a three-fold increase in the rate of pathological complete responses among patients with amplification of the topo II α gene, regardless of the p-53 status (pCR = 10% in patients without amplification vs 30% in patients with amplification, relative risk = 0.33, odds ratio = 0.26), and

   b) that there is a four-fold increase in the rate of pathological complete responses among patients with amplification of the topo II α gene and who are p-53 negative (pCR = 10% in patients without amplification vs 40% in patients with amplification, relative risk = 0.25, odds ratio = 0.17).

The study is sized to have an 80% probability of detecting these odds ratios, using two one-sided tests at 2.5% each (for an overall α-level of 5%). Under these assumptions, the following numbers of patients are required, provided that the rate of p-53 mutated tumors will be 50% in this subgroup of ER- HER-2 amplified tumors:

- if patients with topo II α gene amplification represent 30% of all HER-2 positive patients, a total of 158 HER-2 positive patients would be needed (110 without topo II α gene amplification, 48 with amplification);
- if patients with topo α gene amplification represent 40% of all HER-2 positive patients, a total of 134 HER-2 positive patients would be needed (80 without topo II α gene amplification, 54 with amplification);
- if patients with topo II α gene amplification represented 50% of all HER-2 positive patients, a total of 126 HER-2 positive patients would be needed (63 without topo II α gene amplification, 63 with amplification).

2. In **HER-2 negative** patients, the two hypotheses of interest are:

   a) that there is a two and a half-fold increase in the rate of pathological complete responses among patients with topo II α protein overexpression, regardless of the p-53 status (pCR = 10% in patients without topo II α protein overexpression vs 25% in patients with topo II α protein overexpression, relative risk = .40, odds ratio = .33), and

b) that there is a three and a half-fold increase in the rate of pathological complete responses among patients with topo II α protein overexpression and who are p-53 negative (pCR = 10% in patients without topo II α protein overexpression vs 35% in patients with topo II α protein overexpression , relative risk = .29, odds ratio = .21).

The study is sized to have an 80% probability of detecting these odds ratios, using two one-sided tests at 2.5% each (for an overall α-level of 5%). Under these assumptions, the following numbers of patients are required, provided that the rate of p-53 mutated tumors will be 50% in this subgroup of ER- HER-2 non amplified tumors:

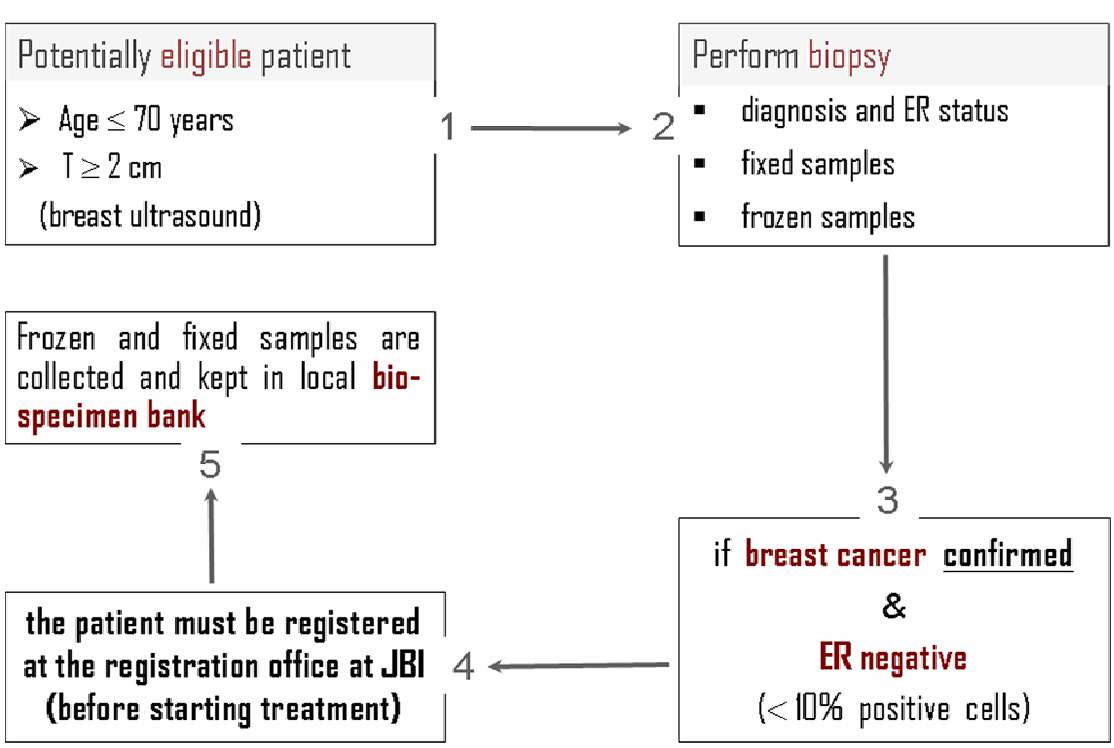
- if patients with topo II α protein overexpression represent 30% of all HER-2 negative patients, a total of 224 HER-2 negative patients would be needed (156 low topo II α protein content, 68 topo II α protein overexpression).
- if patients with topo II α protein overexpression represent 40% of all HER-2 negative patients, a total of 204 HER-2 negative patients would be needed (122 low topo II α protein content, 82 topo II α protein overexpression).
- if patients with topo II α protein overexpression represent 50% of all HER-2 negative patients, a total of 202 HER-2 negative patients would be needed (101 low topo II α protein content, 101 topo II α protein overexpression).

✓ No matching is foreseen for any clinical or biological feature, but the comparison of response rates will be stratified for tumor size using the Mantel-Haenszel approach.

✓ An interim analysis will be performed after the half of the total target accrual, i.e. after inclusion of 180 patients.

III.8.8 Study registration and logistics

The following schema summarizes procedures for study registration:



⇨ *If the patient is eligible, neo-adjuvant epirubicin will have to be started within three weeks from the date of patient's registration in the trial.*

III.8.9 Ethics and informed consent

The protocol and the informed consent statement has to be approved by the ethics committee of each participating center. Progress reports and serious adverse events, life threatening problems or deaths have to be reported to the ethics committee.

Only patients who will have given written informed consent will be eligible for participation in the study. The study will be conducted according to the ethical principles reported in the declaration of Helsinki.

III.8.9.1 Case report forms (CRFs), record retention, SAE report, data management and statistical analysis

Specific CRFs will be available for this study and will have to be regularly filled in during the study conduction. CRFs and all original data should be readily available for review during scheduled monitoring visits. Any data to be recorded directly on the CRF will be considered to be the source data. Copies of all pertinent information will be retained by the investigator for a period of at least 15 years from study completion.

Serious adverse events (SAE) during neoadjuvant therapy have to be reported to Jules Bordet Institute. Only unexpected and rare SAE have to be reported within 24 hours. Other SAE have to be reported within one week. For example, febrile neutropenia has to be reported within one week whereas cardiac toxicity has to be reported within 24 hours. Data will be centralized at the Jules Bordet Institute in Brussels, where data management will be performed.

The statistical analysis will be performed by Dr M. Buyse (International Institute for Drug Development, Brussels).

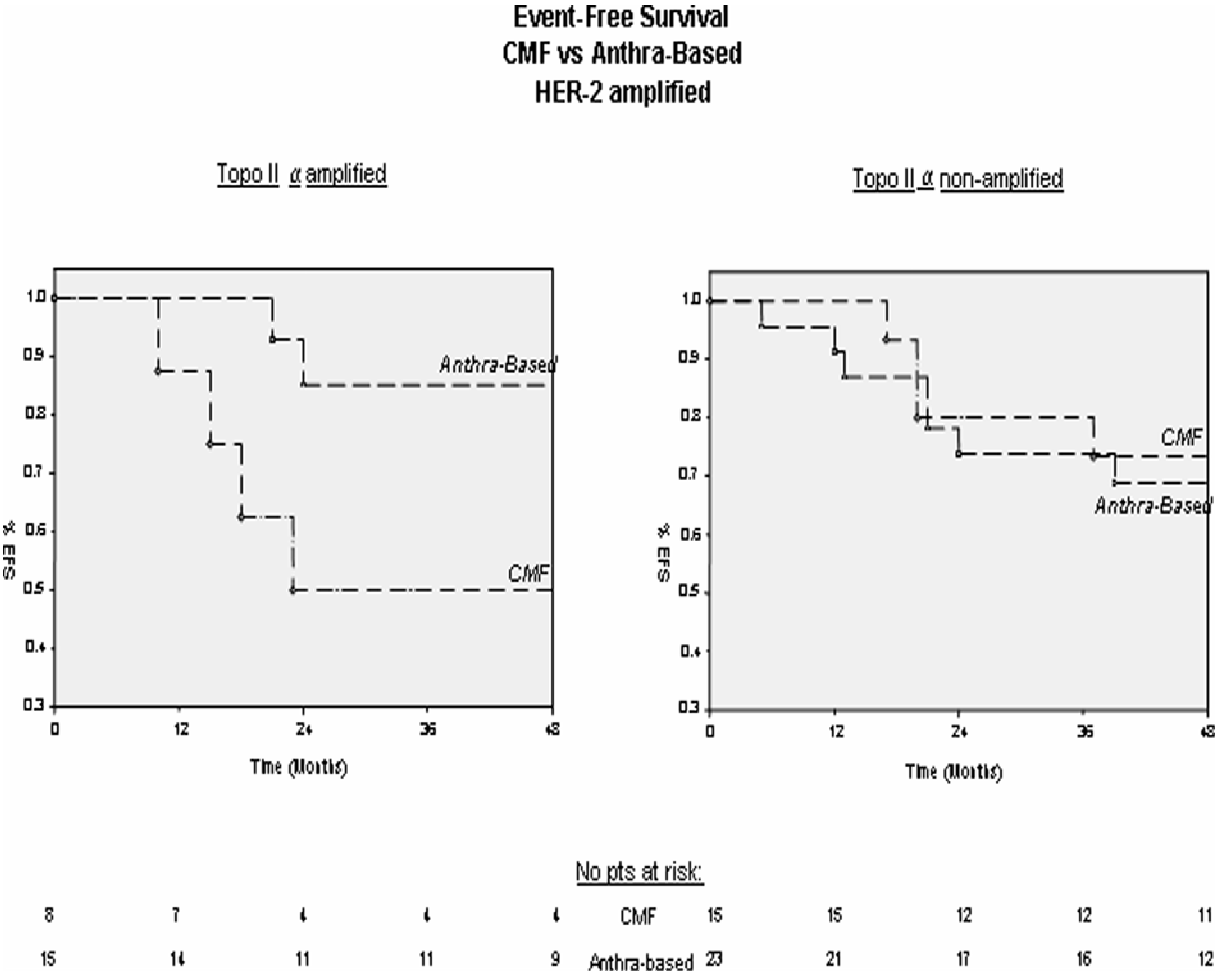
III.8.9.2 Publication policy

It is understood that there is an obligation to provide the Jules Bordet Institute with complete data obtained during the study. The information obtained from the clinical study may be disclosed to regulatory authorities, other investigators, corporate partners, or consultants as required.

It is anticipated that the results of this study will be presented at scientific meetings and/or published in a peer reviewed scientific or medical journal. No publication of the study will be made without the approval of the Jules Bordet Institute. Jules Bordet Institute will provide any prepared abstract or manuscript to the investigators for review at least 15 days prior to submission to a publisher. Investigators who will have contributed more than 5% of eligible patients will be included as co-authors of the main publication. For abstracts the cut-off will be 10%.

Appendix 1

Event-free survival comparison between CMF and anthracyclines (anthra) - based therapy in HER-2 amplified-topo II α amplified patients and HER-2 amplified-topo II α non amplified patients.



Appendix 2

INSTRUCTIONS: After freezing in liquid nitrogen, place the sampling tube with this form in the zip lock bag and transfer everything into a –80°C freezer (see Biospy sampling and freezing procedure booklet).

TOP trial/ TUMOR SAMPLE FORM

Investigator's Name:_____ Investigator's No: |__|__|

Investigator's Institution:_____

Investigator's country:_____

Name of person completing this form: _____ Signature:_____

Date sample is taken: |__|__|__|__|__|__|__|__|

Patient Initials	Date of Birth	Patient Hospital Chart No
|___|___|___|	|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|__|__|__|__|

Appendix 3

Gently dip 2/3 of the Versandtub
in the liquid nitrogen
(liquid nitrogen must not be in contact
with the Tissue tek at that time !)
When the Tissue Tek is solid (2-3 min)
place the Versandtube completely
In the liquid nitrogen.

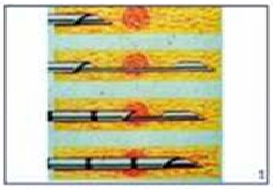
When tottaly frozen,
place the 2 Versandtubes into the zip-lock bag
with the tumor sample form ...

... and transfer the closed zip-lock bag
Into a -80°C freezer.

Sampling procedure

A. 2 trucut biopsies to be FIXED
- To be fixed according to each centre policy
- 1 trucut biopsy for local histopathological diagnosis
- 1 trucut biopsy for TOP trial

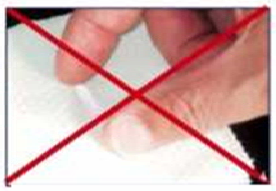
B. 2 trucut biopsies to be FROZEN
- to be frozen according to the procedure described next page
- 2 trucut biopsies for TOP trial



4 trucut biopsies with a 14G needle (NOT with 16G or 18G)

No incisional biopsy is allowed

To be placed on the dry compress and snap frozen IMMEDIATELY according to the freezing procedure.



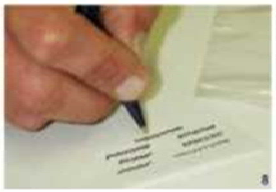
Do not touch the biopsy without sterile gloves.

Do not add physiological serum.

Freezing procedure

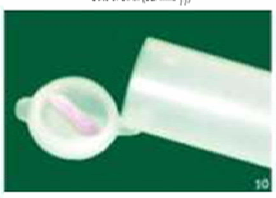
The pathologist must use the supplies provided by the J. Bordet Institute, Brussels: Labels, Versandtubes, Tissue tek, etc.

Complete the tumor sample form legibly



With the water-proof pen, the Pathologist writes on the cryogenic tube label:
Sample data (dd/mm/yy)
Patient initials (3 digits)
Date of birth (dd/mm/yy)

Apply the label on the Versandtube
before freezing



Place each biopsy specimen on the inside of the tube cap (one specimen per tube) and close the tube

Fill 3/3 of the closed Versandtube with Tissue tek.

Appendix 4. Summary of pegfilgrastim characteristics

Pegfilgrastim (Neulasta®)will be administered at a fixed dose of 6 mg (0.6 mL of a 10 mg/mL solution)as a single subcutaneous injection on day 2 after the administration of Epirubicin in the dose-dense arm (every two weeks).

- ***Packaging and Formulation.*** Pegfilgrastim is packaged as a carton box with 1 prefilled syringe (0.6 ml injectable volume per prefilled syringe). Pegfilgrastim is a clear, colourless, sterile protein solution (10 mg/mL). The buffered solution (pH4.0) contains 10 mM sodium acetate, 0.004% polysorbate 20, and 5% sorbitol.

- ***Storage.*** The supplied investigational product must be stored at 2 to 8° C. Exposure of the material to excessive temperature above or below this range should be avoided. Do not allow drug to freeze, and do not use if contents freeze in transit or in storage. Product must be stored in a secured refrigerator in an area with a restricted area (Refer to current SPC).

Appendix 5: TOP-trial Case Report Forms (CRFs) [v.23, August 2006]

The Trial of Principle (TOP trial)

Case Report Form

The Trial of Principle

PROSPECTIVE EVALUATION OF TOPOISOMERASE II ALPHA GENE AMPLIFICATION AND PROTEIN OVEREXPRESSION AS MARKERS PREDICTING THE EFFICACY OF EPIRUBICIN IN THE PRIMARY TREATMENT OF PATIENTS WITH BREAST CANCER

Registration Number: I__|__|__|__|__|__|
Patient initials: I__|__|__|
Institution number: I__|__|
Responsible physician: _____

Instructions: Please fill out the CRF and fax it to the J. Bordet Institute at the fax no. +32 2 5413090

CRF Index

The Trial of Principle (TOP trial)

The Trial of Principle (TOP trial)

Schedule of Assessment

Mandatory Exams ([a] if consent)	**Baseline** **< 28 days before 1st infusion**	**Epirubicin Treatment period**	**Post-Epirubicin Treatment**
Medical history	X		
Physical examination + clinical tumor assessment	X	X	X
Breast biopsy (TRU-CUT) + measurement of hormone receptors	X		
Serum Sample [a]	X[1]	X[2]	X[3]
Whole Blood Sample [a]	X[4]		
Hematology and Biochemistry Red blood cells, Hb, Platelets WBC ANC Total Bilirubin Serum Creatinine GOT/GPT Alkaline Phosphatase	X	X°	X
ECG	X		
LVEF (US or MUGA)	X		
Chest X-Ray	X		
Bone Scan*	X		
Liver Ultrasound	X		
Bilateral Mammography	X		X
Breast Ultrasound	X		X
Informed consent	X		

° Every 21 days, before each cycle of chemotherapy
*Confirmatory X-Ray or CT-Scan in case of hot spots
1 at diagnosis
2 after the first chemotherapy cycle and at surgery time
3 just before adjuvant therapy and just before the last adjuvant therapy
4 before starting treatment

The Trial of Principle (TOP trial)

PLEASE COMPLETE THIS TOGETHER WITH THE REGISTRATION CHECK LIST AND FAX TO: Translational Research Unit- J.Bordet Institute, Belgium
Fax number: 32.2.541.30.90

TOP trial/ PATIENT REGISTRATION FORM

TO BE FILLED IN BY THE INVESTIGATOR

Investigator's Name:_____ Investigator's No: |__|__|

Investigator's Institution:_____

Investigator's country:_____

Name of person completing this form: _____ Signature:_____
Fax Number:|_____|

Date of patient registration: |__|__|__|__|__|__|__|__| **Dose Dense:** ☐ **YES** ☐ **NO**

Patient Initials	**Date of Birth**	**Date treatment planned**
|___|___|___|	|__|__|__|__|__|__|__|__|	|__|__|__|__|__|__|__|__|

TO BE FILLED IN BY THE TRANSLATIONAL RESEARCH

PATIENT INCLUSION

PATIENT ELIGIBLE: ☐ **YES**

☐ **NO, reason:**_____

Date Inclusion: |__|__|__|__|__|__|__|__|

Patient Number: |___|___|___|___|___|

Comments:_____
_____
_____

Translational Research Unit signature:_____

_____

Date: ___/___/_____ Investigator'signature:_____

Top Trial; CRF v. 23 August 2006 Page 4

The Trial of Principle (TOP trial)

Registration checklist

INSTRUCTIONS: *Eligible patients should be registered by faxing this form, signed by the investigator, to the Translational Research Unit of the J.Bordet Institute +32 2 541 30 90. If all eligibility criteria are met a confirmation of registration will be faxed to you with the registration number.*

Registration Number: I__|__|__|__|__|__I Patient initials: I__|__|__|

Date of Birth: _ _ /_ _ / _ _ _ _ (day/month/year) Patient Hospital Chart Number: _____

Institution: _____Fax number: _____

Institution number: I__|__| Responsible physician: _____

Eligibility criteria

Inclusion criteria	***Yes***	***No***
1. Histologically-confirmed operable breast cancer (either operable, or locally Advanced or inflammatory)	☐	☐
2. Age ≤ 70 years	☐	☐
3. Female patient	☐	☐
4. Tumor size ≥ 2 cm at the ultrasound examination	☐	☐
5. ER-negative tumors, defined according to immunohistochemistry (i.e. < 10% of positive cells after immunostaining)	☐	☐
6. In case of multifocal or multicentric tumor: fixed and frozen samples obtained for each nodules and ER-negativity of each nodule confirmed NA ☐	☐	☐
7. Fixed and frozen samples from the primary tumor, obtained before treatment with epirubicin, must be available for evaluation of biological markers (topo II α gene, p-53 gene, cDNA microarrays)	☐	☐
8. Written informed consent before study registration	☐	☐
9. Performance status ≤ 1 (ECOG Scale)	☐	☐
10. ANC ≥ 1500/mm3, platelets ≥ 100.000/mm3, Hb ≥ 10g/dl Tot. bilirubin and serum creatinine ≤ 1 N, GOT/GPT ≤ 1.5 N, AP ≤ 2 .5 N	☐	☐
11. Normal LVEF by Echocardiography or MUGA scan	☐	☐
12.Negative pregnancy test for all women of childbearing potential. Patient of childbearing potential must implement adequate non-hormonal measures to avoid pregnancy during treatment.	☐	☐ or NA ☐

Exclusion criteria		
1. Metastatic breast cancer	☐	☐
2. Serious medical conditions like :	☐	☐
a) congestive heart failure or unstable angina pectoris, previous history of myocardial infarction within 1 year from study entry, uncontrolled arrhytmias	☐	☐
b) history of significant neurologic or psychiatric disorders	☐	☐
c) Active uncontrolled infection	☐	☐
d) Active peptic ulcer, unstable diabetes mellitus	☐	☐
3. Concomitant contralateral invasive breast cancer		
4. Concurrent treatment with hormonal replacement treatment	☐	☐
5. Concurrent treatment with any other anti-cancer therapy	☐	☐
6. Previous treatment with anthracyclines for breast cancer	☐	☐

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Patient's Characteristics

- Height |__|__|__| (cm)
- Weight |__|__|___,__| (Kg)
- BSA |___,___| (m^2)
- Menopausal status:
  - ☐1 premenopausal (< 6 months since last menstrual period (LMP) and no prior ovariectomy and no estrogen replacement therapy)
  - ☐2 postmenopausal (prior bilateral ovariectomy, or > 12 months since LMP with no prior hysterectomy and not receiving LH-RH analog)
  - ☐3 above category not applicable and < 50
  - ☐4 above category not applicable and ≥ 50
- Significant medical history:
  - ☐1 No
  - ☐2 Yes, please specify below

Disease	Date started (day/month/year)	Date ceased (day/month/year) or Ongoing	
	__/__/____	__/__/____	☐
	__/__/____	__/__/____	☐
	__/__/____	__/__/____	☐
	__/__/____	__/__/____	☐

Primary Breast Cancer

- Date of Trucut Biopsy: __/__/____ (day/month/year)
- Trucut Biopsy identification number: _____
- Side of lesion ☐1 Left ☐2 Right

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Pre-treatment tumor characteristics

- Date of histologic diagnosis (day/month/year)…….. _ _ / _ _ / _ _ _ _
- Estrogen receptor status:
  - ☐1 negative
  - ☐2 positive……I___I % positive cells
  - ☐2 unknown
- Progesterone receptor status:
  - ☐1 negative
  - ☐2 positive……I___I % positive cells
  - ☐3 unknown
- Histopathologic type:
  - ☐1 invasive ductal carcinoma
  - ☐2 invasive lobular carcinoma
  - ☐3 other, specify: _____
- Ductal in situ carcinoma:
  - ☐1 No
  - ☐2 Yes
- Lobular in situ carcinoma:
  - ☐1 No
  - ☐2 Yes
- Histopathologic grade:
  - ☐0 not assessable
  - ☐1 GI (well differentiated)
  - ☐2 GII (moderately differentiated)
  - ☐3 GIII (poorly differentiated)
- T classification (primary tumor)
  - ☐1 T1
  - ☐2 T2
  - ☐3 T3
  - ☐4 T4
- N classification (regional lymph node)
  - ☐1 N0
  - ☐2 N1
  - ☐3 N2
  - ☐4 N3
- M classification (distant metastasis
  - ☐1 = MX
  - ☐2 = M0
  - ☐3 = M1

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Tumor Assessment Baseline Evaluation

- Primary tumor

Method:	Date	Measurements
Clinical examination	|__|__|__|__|__|__|__|	|__|__|__| (mm)
Ultrasound	|__|__|__|__|__|__|__|	|__|__|__| (mm)

- Primary tumor 2 if multifocal or multicentric lesions ☐ NA

Method:	Date	Measurements
Clinical examination	|__|__|__|__|__|__|__|	|__|__|__| (mm)
Ultrasound	|__|__|__|__|__|__|__|	|__|__|__| (mm)

- Lymph node 1

Involved ☐ 1 No ☐ 2 Yes

Method:	Date	Measurements
Clinical examination	|__|__|__|__|__|__|__|	|__|__|__| (mm)
Ultrasound	|__|__|__|__|__|__|__|	|__|__|__| (mm)

- Lymph node 2

Involved ☐ 1 No ☐ 2 Yes

Method:	Date	Measurements
Clinical examination	|__|__|__|__|__|__|__|	|__|__|__| (mm)
Ultrasound	|__|__|__|__|__|__|__|	|__|__|__| (mm)

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__| **Patient initials:** |__|__|__|

Administration of Epirubicin

Cycle Number: ☐1 ☐2 ☐3 ☐4 ☐5 ☐6

- Was treatment dose reduced during this cycle
  - ☐$_1$ No
  - ☐$_2$ Yes, please specify reason
    - ☐$_1$ hematological toxicity
    - ☐$_2$ infection
    - ☐$_3$ non-hematological toxicity
    - ☐$_4$ other: specify:_____

- Was treatment dose delayed during study?
  - ☐$_1$ No
  - ☐$_2$ Yes, please specify reason
    - ☐$_1$ hematological toxicity
    - ☐$_2$ infection
    - ☐$_3$ non-hematological toxicity
    - ☐$_4$ other: please specify:_____

- Date of administration of Epirubicin _ _ / _ _ / _ _ _ _
- Total dose administered _ _ _ _ mg
- If dose dense, was GCSF given according to the protocol during cycle? ☐$_1$ No ☐$_2$ Yes ☐$_3$ NA
  - Date of administration _ _ / _ _ / _ _ _ _
  - Total dose administered _ _ _ _ mg

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__| Patient initials: |__|__|__|

Toxicity

Cycle Number : ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐ Check if **NONE**

Adverse event	Grade (1-4)	Start date or ongoing	Stop date or ongoing	Serious Yes*/No	Relation to study medication Yes/No
Allergy					
Gastrointestinal					
Nausea					
Vomiting					
Diarrhea					
Stomatitis					
Pulmonary					
Cough					
Dyspnea					
Pleural effusion					
Neurological					
Neuropathy-sensory					
Neuropathy-motor					
Cardiovascular (arrhytmia)					
Arrhythmia					
Cardiovascular (general)					
Edema					
Hypotension					
Skin					
Alopecia					
Nail disorders					
Constitutional symptoms					
Asthenia					
Fever					
Pain					
Myalgia					
Arthralgia					
Sexual / reproductive function					
Irregular menses					
Infection					
Infection w/o neutropenia:					
Infection					
Other:					
Other:					
Other:					
Other:					
Other:					
Other:					
Other:					
Other:					
Other:					
Other:					

***if an adverse event is SERIOUS, a SAE form must be filled out and faxed to Jules Bordet Institute + 32 3 541 30 90**

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Primary Treatment Completion

- Total number of Epirubicin cycles given: |_____|
- Dose dense ☐1 No ☐2 Yes
- Cumulative dose Epirubicin administered: |__|__|__| (mg/m2)
- End of Epirubicin treatment reason:

  ☐1 Received maximum number of cycles as per protocol

  ☐2 Disease progression/relapse during active treatment (fill out Relapse form **and** Tumor Assessment form **if local relapse**)

  ☐3 Death (fill out the Death Form)

  ☐4 Adverse event, specify:_____

  ☐5 Consent withdrawn, specify: _____

  ☐6 Lost to follow-up

  ☐7 Major protocol violation, specify:_____ _____

  ☐8 Other, specify: _____

Date: ___/___/_____ Investigator'signature:_____

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__| **Patient initials:** |__|__|__|

Tumor Assessment: Post-Epirubicin Evaluation

- Primary tumor

Method:	Date	Measurements
Clinical examination	__/__/____	|__|__|__| (mm)
Ultrasound	__/__/____	|__|__|__| (mm)

- Primary tumor 2 if multifocal or multicentric lesions ☐ NA

Method:	Date	Measurements
Clinical examination	__/__/____	|__|__|__| (mm)
Ultrasound	__/__/____	|__|__|__| (mm)

- Lymph node 1
  Involved ☐$_1$ No ☐$_2$ Yes

Method:	Date	Measurements
Clinical examination	__/__/____	|__|__|__| (mm)
Ultrasound	__/__/____	|__|__|__| (mm)

- Lymph node 2
  Involved ☐$_1$ No ☐$_2$ Yes

Method:	Date	Measurements
Clinical examination	__/__/____	|__|__|__| (mm)
Ultrasound	__/__/____	|__|__|__| (mm)

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Breast Cancer Surgery

Type of Surgery:	**Date** (day/month/year)
1. Lumpectomy	
2. Quadrantectomy	
3. Mastectomy	
4. Axillary Node Dissection	
5. Others, specify: _____	

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Residual Tumor Characteristics

- Size of the invasive component |__|__|__| X |__|__|__| (mm)
- pT:
  - ☐1 pTx: primary tumor cannot be assessed
  - ☐2 pT0: No evidence of any primary tumor
  - ☐3 pTIS: Carcinoma in situ as sole remaining tumor: intraductal carcinoma or lobular carcinoma in situ or Paget disease of the nipple as all remaining tumor
  - ☐4 pT1: Invasive tumor of 2 cm or less in its greatest dimension
  - ☐5 pT2: Invasive tumor more than 2 cm but not more than 5 cm in its greatest dimension
  - ☐6 pT3: Invasive tumor more than 5 cm in its greatest dimension
  - ☐7 pT4: Invasive tumor of any size with direct extension to chest wall or skin
- Multifocal Tumor ☐1 No ☐2 Yes ☐3 NA
- Multicentric Tumor ☐1 No ☐2 Yes ☐3 NA
- Surgical margins:
  ☐1 Negative (≥ 1 mm)
  ☐2 Close (< 1 mm)
  ☐3 Involved
- Histopathologic type:
  ☐1 invasive ductal carcinoma
  ☐2 invasive lobular carcinoma
  ☐3 other, specify: _____
- Ductal in situ carcinoma:
  ☐1 No
  ☐2 Yes
- Lobular in situ carcinoma:
  ☐1 No
  ☐2 Yes
- Histopathologic grade:
  ☐1 not assessable
  ☐2 GI (well differentiated)
  ☐3 GII (moderately differentiated)
  ☐4 GIII (poorly differentiated)

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Regional Lymph Nodes

- Number of resected axillary lymph nodes |_____|
- Number of positive axillary lymph nodes |_____|

- pN:
  - ☐1 pNx: regional lymph nodes cannot be assessed
  - ☐2 pN0: no regional lymph nodes metastasis
  - ☐3 pN1: metastasis to movable ipsilateral axillary node(s)
  - ☐4 pN2: metastasis to ipsilateral lymph node (s) that are fixed to one another or to other structures
  - ☐5 pN3: metastasis to ipsilateral internal mammary lymph node (s)

Final Response Assessment

- pCR (pathological complete response (pT0 or pTIS and pN0)
  - ☐1 No
  - ☐2 Yes: ☐ pT0
    ☐ pTis
    ☐ pN0

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__|

Post-Surgery Treatments

- Radiotherapy ☐1 No ☐2 Yes
- Hormonal Treatment ☐1 No ☐2 Yes, please specify:
  - ☐ Tamoxifen
  - ☐ Aromatase inhibitor, please specify: _____
  - ☐ LHRH analog
  - ☐ Other, please specify: _____
- Adjuvant Chemotherapy ☐1 No ☐2 Yes, please specify:

  ☐ Docetaxel, please specify below:
  Total number of Docetaxel cycles given: |_____|
  Cumulative dose Docetaxel administered: |__|__|__| (mg/m2)

  ☐ Other, please specify_____
  Total number of cycles given: |_____|
  Cumulative dose administered: |__|__|__| (mg/m2)
  Regimen _____
- Adjuvant Herceptin ☐1 No ☐2 Yes, please specify:
  - ☐ In context of HERA trial ☐1 No ☐2 Yes
  - ☐ HERA CRF number: |__|__|__|__|__|__|
- Other Adjuvant Treatments ☐1 No ☐2 Yes
  In context of a trial ☐1 No ☐2 Yes
  If yes, please specify which trial: _____

The Trial of Principle (TOP trial)

Registration Number: |__|__|__|__|__|__|__| **Patient initials:** |__|__|__| **FU No.** |__|__|

Follow up

(Please fax this form every 4 months during the 2 first years of follow up and every 6 months after, up to 5 years of follow up)

Follow up (tick the appropriate box)

☐ 4 months	☐ 2.5 years
☐ 8 months	☐ 3 years
☐ 1 year	☐ 3.5 years
☐ 1 year & 4 months	☐ 4 years
☐ 1 year & 8 months	☐ 4.5 years
☐ 2 years	☐ 5 years

- Follow up date: (day/month/year) _ _ / _ _ / _ _ _ _
- Are there any changes since previous assessment?
  - ☐$_1$ No
  - ☐$_2$Yes, please specify:
    - ☐$_1$ Breast Cancer Relapse (Please complete Relapse section below)
    - ☐$_2$ Second Primary Malignancy (Please complete the SPM section below)
    - ☐$_3$ Death (Please complete the death section below)

Relapse

	Specify	Date of recurrence (day/month/year)
1. Local recurrence		
2. Regional recurrence		
3. Distant recurrence		

Second Primary Malignancy

- Date of second primary malignancy: (day/month/year) _ _ / _ _ / _ _ _ _
  Site: ☐ Controlateral breast
  ☐ Other, specify: _____

Death

- Date of Death: (day/month/year) _ _ / _ _ / _ _ _ _
- Cause of Death
  - ☐$_1$ Progression of disease
  - ☐$_2$ Adverse event; specify: _____
  - ☐$_3$ Other; specify: _____

The Trial of Principle (TOP trial)

SERIOUS ADVERSE EVENT FORM Page 1

Report Initial

Follow- up → Follow-up N° _ _ _

Date of Report (dd/mm/yyyy) _ _/_ _/_ _ _ _

Center number : _____ Institution : _____

Investigator : _____

Patient Information

Initials : _ _ _ Study Subject number : _ _ _ _ _ Date of birth (dd/mm/yyyy) : _ _/_ _/_ _ _ _

Sexe: M F Weight : _____ Kg or UNK Height : _____ cm or UNK

Event Details

Event	Onset date	Date resolved	CTC Grade (1 to 4)	Serious outcome	Relationship to study drug	Action taken regarding study drug	Outcome
1			|__|	|__|	|__|	|__|	|__|
2			|__|	|__|	|__|	|__|	|__|
3			|__|	|__|	|__|	|__|	|__|
4			|__|	|__|	|__|	|__|	|__|

Use the following table to fill the Event details Table

Serious outcome	**Relationship** :	**Action Taken**	**Outcome** :
1 = death	1 = definitely	1 = None	1 = resolved
2 = life threatening	2 = Probably	2 = Reduced	2 = Ongoing
3 = hospitalization	3 = Possibly	3 = Interrupted	3 = Improved
4 = disability	4 = Unrelated	4 = Discontinued	4 = Worsened
5 = congenital anomaly			5 = Unchanged
6 = other			

Investigational Agent

Drug	Regimen	Cycle Number	Total daily dose	Date first dose	Date last dose

Lab Tests & Exams

Test	Result

The Trial of Principle (TOP trial)

SERIOUS ADVERSE EVENT FORM TOP TRIAL Page 2

Concomitant **Medications**							
Medication	Dose	Route	Indication	Started dd/mm/yy	Stopped dd/mm/yy	Used to treat the event	
						No	Yes
						No	Yes
						No	Yes
						No	Yes
						No	Yes

Description of event :
Was the suspected drug reintroduced? Yes No
If YES, did the event reappear? Yes No

Relevant Medical History
□ Alcoholism
□ Tabagism
□ Allergies
□ Other:

Physician Name :
Physician Phone number:

IV. The ACGT In Silico Oncology Research

IV.1 Clinical Validation of the *In Silco* Oncology Simulation Models

The advancement and clinical validation, adaptation and utilization of *in silico* (computational) oncology is one of the major targets of the ACGT project. The aim is to provide clinicians with a decision support tool able to simulate within defined reliability limits the response of a solid tumour to therapeutic interventions based on the individual patient's data. The treatment effects on the normal tissues will also be taken into account even in considerably less detail. An intermediate goal of this action is to provide researchers with a versatile platform for integrating experimental and clinical knowledge and performing exploratory experiments *in silico* (on the computer). Therefore, the proposed system is expected to become a prototype *multi-level* cancer biology *integrator*.

The constituent simulation models will be based on the novel, essentially "top-down" modeling approach developed by the In Silico Oncolog Group, ICCS, National Technical University of Athens. Although extensive exploitation of relevant previous work done by ACGT members will take place, large scale extensions and modifications will be implemented in order to cope with the particularly high demands and intricacies of the two clinical cases addressed by ACGT i.e. nephroblastoma (Wilm's tumor) and breast cancer. To this end a computational system denoted by the specially coined term "Oncosimulator" will be developed.

As clinical validation of the "Oncosimulator" will be based on the two clinical trials incorporated in ACGT (nephroblastoma SIOP 2001/GPOH and breast cancer TOP trial), the term "*In Silico* Oncology trial" which is sometimes used in the ACGT context actually refers to a *"metatrial"* i.e. a validation procedure aiming at checking and optimizing a complex simulation system through the *observation* of the *time course* of the corresponding physical system's behaviour (here the tumour). It is pointed out that the design and implementation of clinical trials in order to validate, adapt and optimize tumour behaviour models is a worldwide novelty.

The *In Silico* Oncology clinical test aims at validating, optimizing and clinically adapting the "Oncosimulator" i.e. the simulation model of tumour response to chemotherapy to be developed within the frame of ACGT.

In the following a brief outline of the *generic* "Oncosimulator" concept and system is given. It should be noted that slight *modifications* and *adaptations* of it have been made for the particular two branches of the *In Silico* Oncology trial.

IV.1.1 The "Oncosimulator" (*In Silico* Scenario – IS-S)

The "Oncosimulator" will function as shown in Figure 14 (next page).

IV.1.1.1 Combining Clinico-Genomic/Proteomic data.

The tumour biopsy material and blood samples are carefully collected and transported to the DNA microarray facility where the gene expression is obtained. Subsequently, a cancer- and patient specific "gene-protein network" of the tumour is identified based on the gene expression of the particular specimen and the gene clustering, classification and gene selection for each subtype of the tumour considered. Perturbations suggested by molecular data sets are introduced and an estimation of the radiobiological (LQ α and β) and pharmacodynamic (cell survival constant for particular drugs) parameters takes place based on the identified gene-protein network. Concerning e.g. breast cancer, the pharmacodynamic parameters are evaluated depending on the status and expression of critical genes such as topo II α, p53 etc. More generally, if a sequence of molecular events leads to e.g. apoptosis

as a response to irradiation or chemotherapy, a rough semi-quantitative estimation of the radiobiological / pharmacodynamic parameters as variations about their mean values reported in literature can be made. A more quantitative evaluation can be achieved using the patient data to be collected and applying multiple parameter adaptation methods such as genetic algorithms or neural networks.

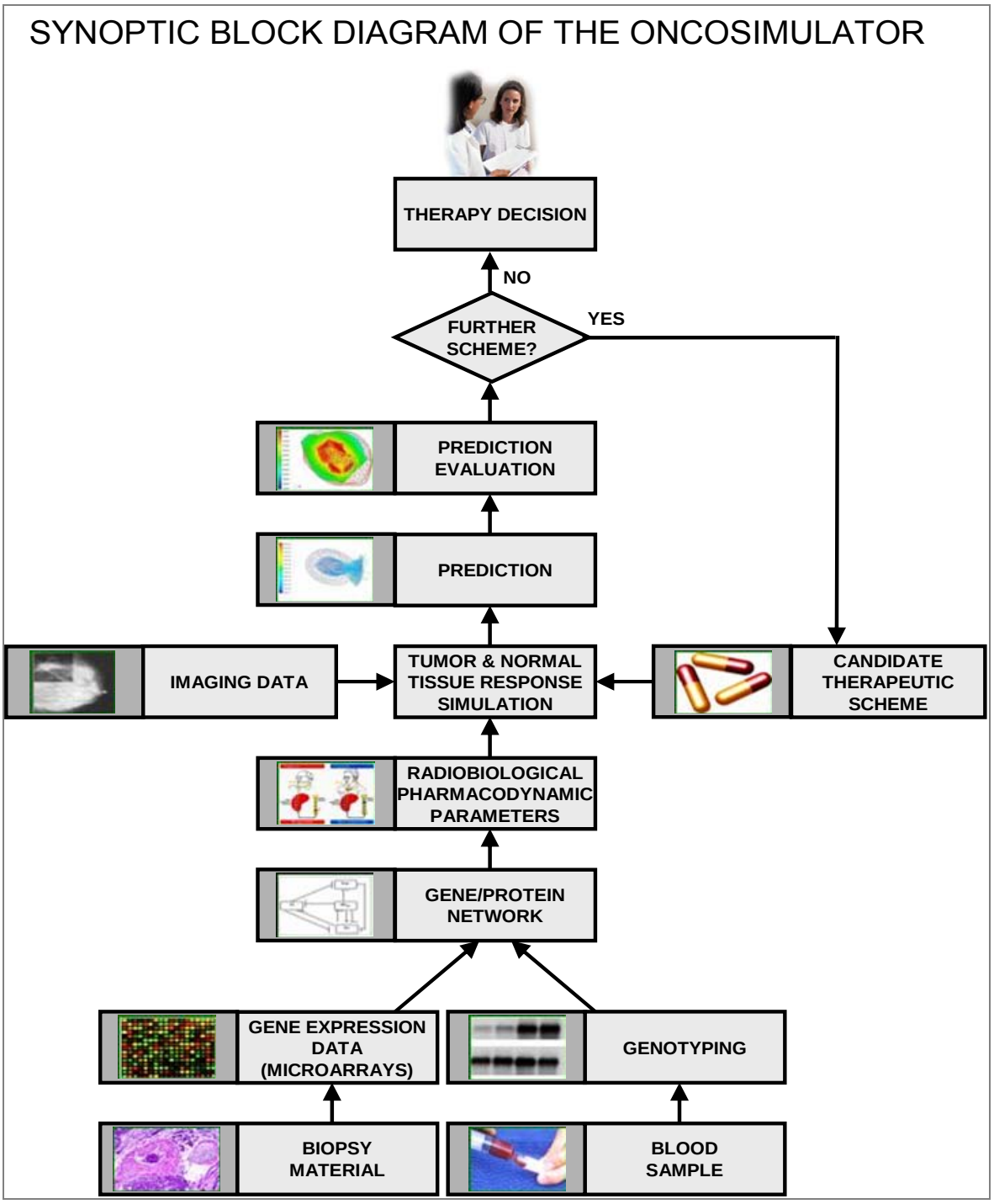


Figure 14. A synoptic block diagram of the "Oncosimulator"

IV.1.1.2 Imaging data

The imaging data (e.g. CT, MRI, PET, etc.) are introduced into the simulation model and a candidate radiotherapeutic or chemotherapeutic scheme is defined. The output of the simulation run which is the prediction of the tumour and critically affected normal tissue response to the treatment scheme is then evaluated by the supervising doctor. If a further scheme is to be tested *in silico*, the simulation run is repeated with the same imaging and radiobiological/pharmacodynamic data as previously. In the end, this multiscale modelling platform serves as a generic "decision-support system". That is the physician makes his or her final decision on the selection of the most promising therapeutic scheme by taking into account both, the predicted outcomes of all simulated regimens as well as his or her own medical knowledge and expertise. This innovative computational platform therefore does not intend to replace the physicians' input but to add the possibility to investigate the impact of specific treatment-induced perturbations over several orders of magnitude – which currently is impossible with conventional imaging methods alone.

The 4D (Monte Carlo – cellular automaton) computer simulation model will be mainly based on the imaging (e.g. ultrasound, CT, MRI, PET), the histopathologic (e.g. exact histological type of cancer) and the molecular (e.g. gene and protein expression) data of the patient. The latter will be processed by appropriate gene networks in order to obtain patient individualized corrections of the mean radiobiological and pharmacodynamic parameters pertaining to the specific tumour type. Such corrections may for example represent increased apoptosis. Other prognostic factors might also be taken into account. A discretization mesh will be superimposed upon the anatomical region of interest which includes the tumour and the adjacent normal tissues according to the imaging data.

The most critical biological phenomena (e.g. metabolism, cell cycling, geometrical growth or shrinkage of the tumour, cell survival following irradiation or chemotherapeutic treatment, necrosis, apoptosis etc.) will be spatiotemporally simulated within each geometrical cell of the mesh.

Due to the extremely large number of cells constituting an *in vivo* tumour, cells will be grouped in *equivalence classes* based on their cell cycle state within each geometrical cell of the mesh. The effect of irradiation on the cell level of biocomplexity will be based on the linear quadratic (LQ) model whereas that of each chemotherapeutic session on the pharmacokinetics and pharamacodynamics of the drugs and their combinations considered.Effective inclusion of the above mentioned elements has already been achieved in the glioblastoma multiforme simulation model developed by the ACGT partner ICCS – NTUA.

Imaging data before, during and after treatment should be adequately fused (if more than one imaging modalities are to be used concurrently e.g. CT and MRI), segmented, 3D reconstructed and registered so that the treatment outcome can be reliably visualized and quantified. To this end a user-friendly set of software modules will be developed implementing the previously mentioned processes. To meet the increased demands of this application both serial acceleration and parallelization of the code to the highest possible degree will be applied. Furthermore, as a number of different candidate therapeutic schemes for any given patient are to be simulated independently, *grid architecture* will be exploited for concurrent code executions.

Virtual Environments designed to represent 3D (and to some degree also 4D) data and to provide intuitive interactive methods to explore this data will be applied for *the virtual reality visualisation* of both medical images and *in silico* oncology simulation results. The objective to "involve" the researcher more, and bring her/him closer to her/his data in an effort to detect patterns and structures using the researcher's experience, expertise and cognitive abilities.

- Consideration of the anatomical, genetic and other details of nephroblastoma and breast cancer tumours will lead to a refinement and adaptation of the model to the breast cancer case. Extensive checks concerning numerical stability, convergence, etc. will be performed. Parametric validation tests will also be performed before clinical testing, adaptation and validation.
- In addition, a synoptic simulation model of normal tissue response to therapeutic schemes will also be developed in order to assess the normal tissue response to breast cancer therapy regimes.

References

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IV.1.2 Background information (clinical validation of tumour response to therapy simulation)

To the best of our knowledge, up to now there have not been any *specially planned*, *large scale*, *molecular biology enhanced* clinical trials [or more correctly clinical validation procedures] in order to test and adapt mathematical or computational models of tumour response to therapeutic modalities. Therefore, the present action seems to be a *worldwide novelty*.

On the contrary, in the past years scant small scale efforts to *clinically* validate tumour growth and response to therapy models have in fact appeared in the literature but all have relied on the rather abstract notions of clinical experience, clinical practice, clinical logic or the outcome of clinical trials *not* designed with a particular view to serve as possible testers of such models. Furthermore such efforts have not included massive molecular biology information and therefore they refer rather to *population based mean* tumour behaviour and not to the patient's individualized response. The following articles are representative of such efforts:

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IV.2 Trial objective and purpose of ACGT *In Silico* Oncology

The objective of the trial is to validate, clinically adapt and optimize the "Oncosimulator" for the special cases of nephroblastoma and breast cancer. To this end:

- For **nephroblastoma**: ***the clinical, imaging and molecular data of the patient***
- For **breast cancer**: ***the clinical, imaging, histopathologic and molecular data of the patient***

Following preprocessing will be introduced into the "Oncosimulator" along with the description of the therapeutic scheme (temporal drug administration scheme) to be simulated.

The prediction of the "Oncosimulator" regarding the tumour response as a function of time will be compared with the imaging data at various instants during and after the chemotherapeutic scheme. The outcome of the comparison will be used as an adaptation / optimization feedback for the "Oncosimulator".

IV.2.1 *In Silico* Oncology trial Design

The design of the trial refers to the two constituent "sub-trials" namely the nephroblastoma (Wilm's tumour) and the breast cancer trials. In the following an outline of both "sub-trials" is presented.

IV.2.1.1 The Nephroblastoma Case

A nephroblastoma (Wilm's) tumour consists generally of a mixture of the histological subtypes *blastemal*, *epithelial* and *stromal* in varying proportions. The tumour responsiveness to chemotherapeutic regimes is highly dependent on the relative contribution of each one of the subtypes and obviously on their genetic characteristics. Furthermore *anaplasia* (which may be focal or diffuse) is another factor significant for the prediction of therapeutic outcome. The histology of nephroblastoma (Wilm's tumour) at the time of presentation provided that no biopsy takes place [as is the case in the SIOP 2001/GPOH clinical trial] is unknown. An indirect way of determining would be of paramount importance in order for the clinician to judge whether or not a particular patient would benefit from chemotherapy. N. Graf has suggested that serum antibody profiling (termed "the antibody scenario") may be used as a surrogate indicator of the actual cell type composition of the tumour.

Based on the previous reasoning the following **clinical scenario** will be implemented within the frame of both ACGT workpackages WP8 and WP12. It is pointed out that for reasons of simplicity and better control only unilateral tumours without nephrogenic rests and metastasis will be considered.

- **Data Collection (**USAAR)**.** After presentation of the patient to the clinical institution, collection of the following data takes place (see also the attached SIOP 2001/GPOH case report form)

- **Clinical Data**
  - Age
  - Sex
  - Weight
  - Height
  - Syndromes (WAGR, Denys-Drash, Beckwith-Wiedermann)
  - Family history

- **Imaging Data (**baseline: just before chemotherapy start)
  - CT (DICOM) and/or MRI (DICOM) and/or ultrasound (DICOM)]
  - Three ellipsoidal axes of the tumour.
  - Delineation of the necrotic, cystic, hemorrhagic and solid tumour regions on the tomograhic slices.

- **Molecular Data**
  - Profiling of antibodies to tumour antigens (antigen scenario)
    ↓
  - Estimated cell type composition of the tumour
    ↓
  - Estimated tumour cell responsiveness to the drugs under consideration

Recommended Treatment Scheme(s) Data

Description of the recommended scheduling of drugs dose administration. The above mentioned data is entered into the “Oncosimulator” which performs the tumour response to chemotherapy simulation. A rough estimation of the response of representative normal tissues is also made. Therefore, the most probable outcome is predicted.
Based on the “Oncosimulator” prediction (mainly the expected tumour shrinkage) , the clinician judges whether or not the chemotherapy outcome would be beneficial to the patient under consideration by also taking into account his or her logic, expertise and even intuition. Independent of this judgement the patient will always receive preoperative chemotherapy, so that the result of the oncosimulator can be compared with the clinical situation after preoperative chemotherapy. This will be done to evaluate the oncosimulator.

If there will be a perfect correlation between the prediction of tumor response by the oncosimulator and the clinical response to preoperative chemotherapy, in future trials the result of the oncosimulator may be used for stratification of treatment. Meaning that in a patient, where the expected outcome is not judged as beneficial, the patient may proceed directly to surgery without receiving preoperative chemotherapy. Otherwise, the chemotherapeutic scheme is applied on the real patient.

The actual chemotherapy administration schedule is registered. The following examinations are carried out during and after treatment:

- **During chemotherapy**
  - Ultrasound imaging every week (if possible)

- Recording of the 3 tumour ellipsoidal axes

- **After completion of chemotherapy**
  - Profiling of serum antibodies against tumour antigens
  - CT (DICOM) and/or MRI (DICOM) and/or ultrasound (DICOM)
  - Three ellipsoidal axes of the tumour.
  - Delineation of the necrotic, cystic, hemorrhagic and solid tumour regions on the tomograhic slices.
  - Serious Adverse Effects (SAE) concerning hematologic reactions

- **After surgery**
  - Histology (types)

The predicted and the actual **outcome** and **histology** are compared and if they are in significant contradiction an optimization and adaptation loop for the "Oncosimulator" is carried out, otherwise the current checking of the "Oncosimulator" is judged as favourable.

IV.2.1.2 The Breast Cancer case

The following flow chart depicts the suggested data and procedures to be used and applied for the adaptation and optimization of the "Oncosimulator" that will be developed within the frame of WP8 (technologies and Tools for *In Silico* Oncology). The *Test of Principle trial (TOP)* concerning epirubicin will be considered.

- **Data Collection** (IJB)**.** After presentation of the patient to the clinical institution collection of the following data takes place (see also the attached TOP case report form)

- **Clinical Data**
  - Age
  - Sex
  - Weight
  - Height
  - Previous treatments
  - Blood cell counts (BCC) {to monitor adverse effects on normal tissues}
  - ACCESS TO ***ALL*** DATA RECORDED IN THE TOP TRIAL DATA BASES DURING THE PATIENT'S TREATMENT.

- **Imaging Data (**baseline: just before chemotherapy start)
  - Ultrasound (DICOM)
  - Prospectively Somo-vu 3D US images
  - Digital mammography (DICOM) for some cases
  - PET and CT or MRI for certain cases (DICOM)
  - ***Three ellipsoidal axes of the tumour*** (obligatory).
  - Delineation of the necrotic, cystic, hemorrhagic and solid tumour regions on the tomograhic slices.

- **Histopathological & Molecular Data**
  - Histopathological profile (metastatic disease?, tumour cell types etc.)
  - Photographs of HE histopathology slides (MIRAX scan system)
  - Topo IIα gene and protein, HER-2 gene, p53 gene, ***DNA array*** based gene expression profiling of the bioptic material

    ↓
  - Estimated tumour cell responsiveness to the drugs under consideration

Recommended Treatment Scheme(s) Data

Description of the recommended scheduling of drug dose administration. The above mentioned data is entered into the "Oncosimulator" which performs the tumour response to chemotherapy simulation. A rough estimation of the response of representative normal tissues is also made. Therefore, the most probable outcome is predicted.

Based on the oncosimulator prediction (mainly the expected tumour shrinkage) , the clinician judges whether or not the chemotherapy outcome would be beneficial to the patient under consideration by also taking into account his or her logic, expertise and even intuition.

⇨ ONLY AFTER THE ONCOSIMULATOR HAS BEEN CHECKED: In case that the expected outcome is not judged as beneficial, the patient may undergo other therapeutic interventions.

⇨ ONLY AFTER THE ONCOSIMULATOR HAS BEEN CHECKED: Otherwise, the chemotherapeutic scheme is applied to the real patient.

The actual chemotherapy administration schedule is registered.

The following examinations are carried out during and after treatment:

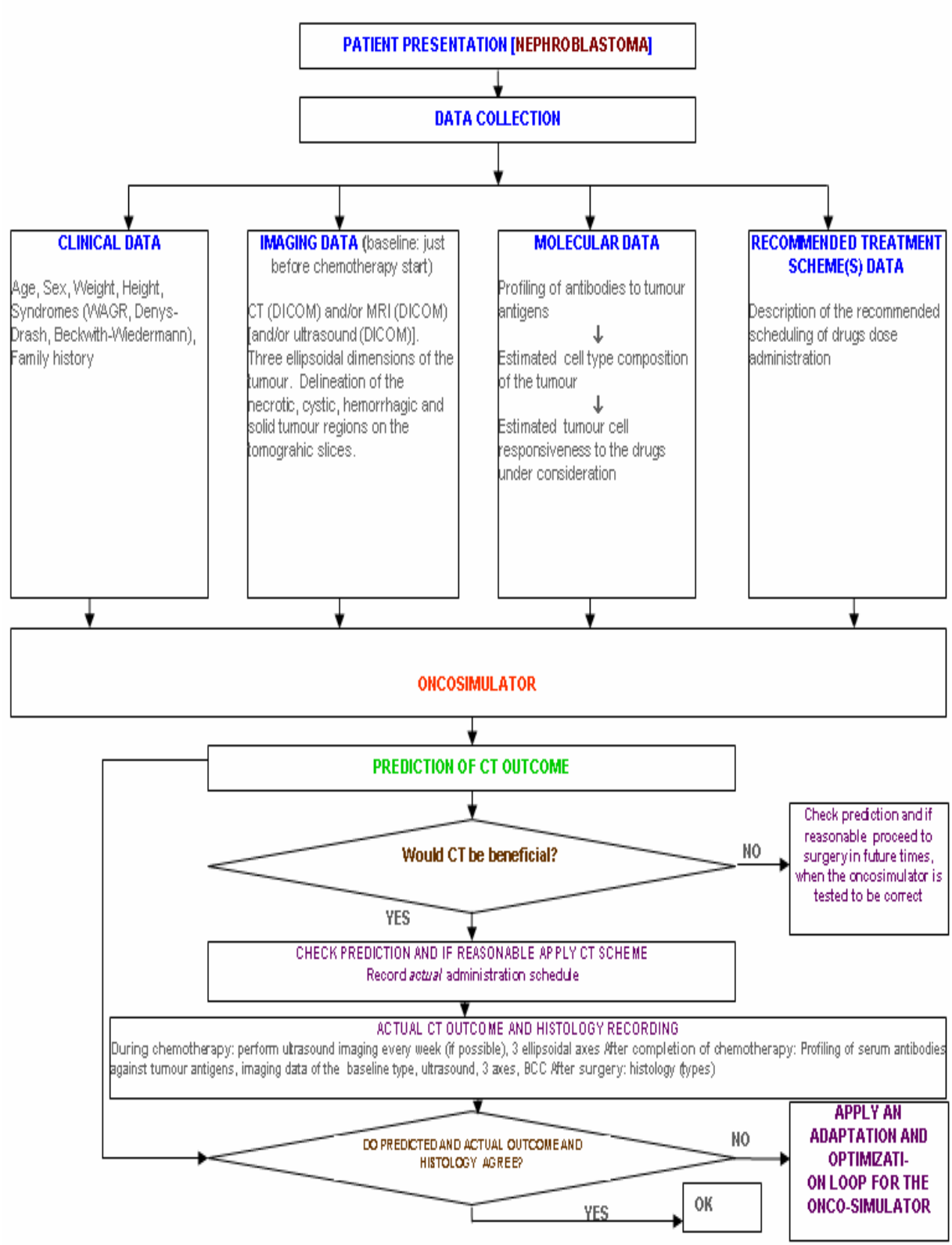
- **During chemotherapy (prospectively)**
  - Ultrasound imaging *after each CT cycle (and preferably on the 1st day of each week of the chemotherapeutic cycle)*
  - Recording of the tumour 3 ellipsoidal axes
- **After completion of chemotherapy**
  - Ultrasound (DICOM)
  - Prospectively Somo-vu 3D US images
  - Digital mammography (DICOM) for some cases
  - PET and CT or MRI for certain cases (DICOM)
  - ***Three ellipsoidal axes of the tumour*** (obligatory).
  - Delineation of the necrotic, cystic, hemorrhagic and solid tumour regions on the tomograhic slices.
  - Blood Cell Counts (BCC)

The predicted and the actual **outcome** are compared and if they are in significant contradiction an optimization and adaptation loop for the "Oncosimulator" is carried out, otherwise the current checking of the "Oncosimulator" is judged as favourable.

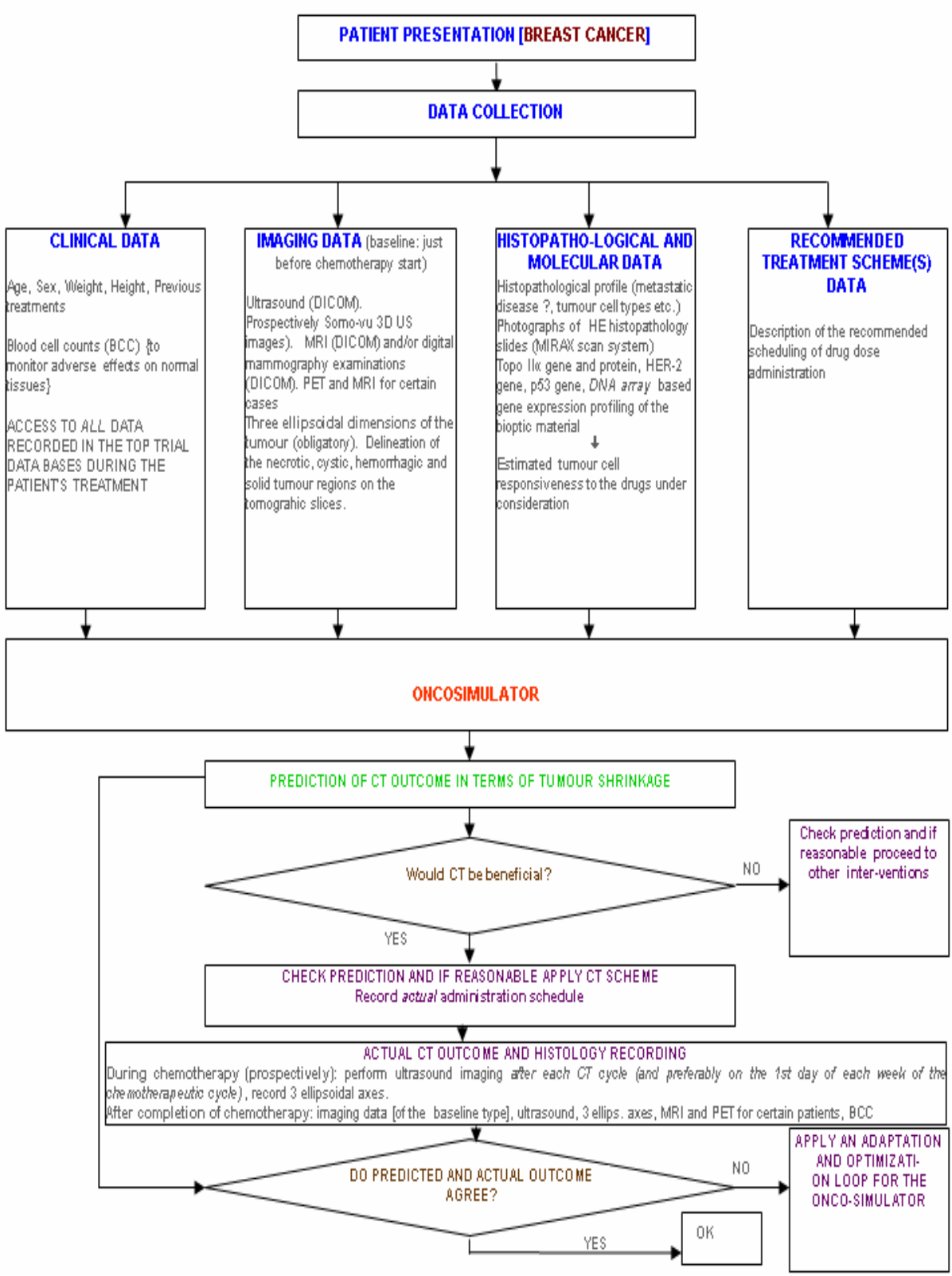
IV.3 In Silico Oncolgy Flow Diagramas

In the following four pages the flow diagrams for the two branches of the *In Silico* Oncology trial namely the nephroblastoma (Wilm's tumour) branch and the breast cancer branch are presented.

IV.3.1 Flow Diagram of the Nephroblatoma Branch of the In Silico Oncology Trial



IV.3.2 Flow Diagram of the Bresat Cancer of the In Silico Oncology Trial



IV.4 Selection, Withdrawal & Treatment of subjects

The selection and eventual withdrawal of subjects will be performed according to the TOP and SIOP 2001/GPOH clinical trial protocols. It is noted that as the *In Silico* Oncology trial is actually a "metatrial" based on the other two ACGT trials, information will be extracted from their data as well as from the additional examinations (mainly ultrasound).

For the special cases where additional examinations entail use of ionizing radiation (PET, CT) written informed consent of the patient will be a prerequisite.

The prescribed TOP and SIOP NB protocols will be applied. *Only* the adjuvant (pre-surgery) chemotherapy treatment will be considered for the needs of the *In Silico* Oncology trial. For specific patients additional examinations will take place provided that informed consent is granted by the patient

IV.5 Assessment of Efficacy & Safety

The assessment of predictive efficacy of the "Oncosimlator" will be primarily based on the imaging data referring to the imageable tumour response to the chemotherapeutic schemes under consideration. Tumour volume will be the fundamental criterion for testing the "Oncosimulator". In specific cases the shape of the tumour and its spatiotemporal metabolic activity distribution will also be used in order to refine the validation criteria.

The only cases that would need further consideration from the safety point of view are those for which CT, PET or CT plus PET will be prescribed. The responsible clinician will take into account the overall irradiation load of the patient, her performance status, other pertinent factors and necessarily the granting or not of the patient's informed consent. Should any of the above factors be not favourable for the performance of the extra examinations, these shall not be undertaken.

IV.6 Statistics

Efforts will be made in order to utilize as many clinical cases as possible in order to validate and adapt/optimize the "Oncosimulator".

A rough estimate of the clinical cases to be considered concerning nephroblastoma would be about 25 per year whereas for breast cancer would be about 30 per year.

As a simulation model offers the possibility of performing a large number of virtual experiments, an optimal exploitation of the clinical data is expected to be reached. Therefore, a set of data is expected to provide more information when used in order to test the 'Oncosimulator" than when used to test simple clinical hypotheses.

The significance of the validation outcomes will be primarily expressed in terms of *p* significance levels.

IV.7 Source Data & Documents - Reporting

Direct access to source data / documents for both the nephroblastoma SIOP 2001/GPOH and the breast cancer TOP trials will be ensured by the generic ACGT infrasctructure. The ACGT ***pseudonymization*** policy compatible with European ethical and legal restrictions will be adopted.

The TOP and SIOP NB case report forms filled in *electronic form* will be used as the reference for the collection and recording of the necessary data. The pseudonymized clinical, imaging, histopathological, molecular and treatment administration data will be transferred from the clinical institutions to ICCS, FORTH and FhG and subsequently to the rest of the partners involved in data processing.

IV.8 Quality Control & Assurance - Ethics

Quality control and quality assurance refer primarily to the clinical part of the trials and therefore pertinent information is to be found in the corresponding descriptions (nephroblastoma SIOP 2001/GPOH and breast cancer TOP trials). Concerning the simulation model codes, *numerical stability* will be ensured especially with regard to the use of pseudorandom number generators (Monte Carlo technique).

As already mentioned the ACGT pseudonymization policy and system will ensure the medical data record privacy. Furthermore, any additional diagnostic examinations will have as a prerequisite the written informed consent of the patient following a thorough consideration of all pertinent factors by the responsible clinician. Only those patients who have not already received considerable ionizing radiation dose will be considered.

IV.9 Financial and insurance matters

The financial and insurance policies adopted by the nephroblastoma SIOP 2001/GPOH and breast cancer TOP trials will apply to the "Oncosimulator" validation process as well. It should be reminded that the *In Silico* Oncology clinical test is a virtual "meta-trial" of the previous two trials rather than a stand alone clinical trial.

IV.10 Publication policy

The publication of any material including scientific papers related to the clinical validation of the "Oncosimulator" to be undertaken within the frame of ACGT will be co-ordinated as follows:

1. For the nephroblastoma branch publication of relevant material will be jointly co-ordinated by University of Saarland and ICCS
2. For the breast cancer branch publication of relevant material will be jointly co-ordinated by Institut Jules Bordet and ICCS

Credit will be given to the involved partners in a way proportional to their contribution in the implementation of the Oncosimulator clinical validation procedure.

IV.11 Supplements

The case report forms of both the nephroblastoma SIOP 2001/GPOH and the breast cancer TOP trials are to be found in the corresponding clinical trials sections of the present report. The detailed description of data to be collected is to be found in sections 3 (Trial objective and purpose) and 4 (Flow diagram).