Clinically Oriented Translational Cancer Multilevel Modelling

Deliverable D2.1

Requirements and specification for the *ContraCancrum* integrated technological platform

Project Number: FP7--IST-*223979*

Deliverable id: D2.1

Deliverable Name: Requirements and specification for the *ContraCancrum* integrated technological platform

Submission Date: 29/01/2009

COVER AND CONTROL PAGE OF DOCUMENT	
Project Acronym:	CONTRACANCRUM
Project Full Name:	Clinically Oriented Translational Cancer Multilevel Modelling
Document id:	D2.1
Document name:	Requirements and specification for the *ContraCancrum* integrated technological platform
Document type	INT
Version:	3
Submission date:	29/01/2009
Editor: Organisation: Email:	Gordon Clapworthy BED gordon.clapworthy@beds.ac.uk

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

ABSTRACT:

This deliverable identifies and characterises the anticipated user population of the ContraCancrum integrated technological platform and the uses to which it will be put. From these, it produces a broad specification for the system which will be further refined in a later deliverable.

KEYWORD LIST: User, specification, requirement

MODIFICATION CONTROL			
Version	Date	Status	Author
1.0	15/12/2008	Draft	All partners
2.0	22/01/2009	Penultimate	All partners
3.0	27/01/2009	Final	All partners

List of Contributors

- all partners contributed widely in the creation of this deliverable

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Publishable Executive Summary

The ContraCancrum, i.e. the Clinically Oriented Translational Cancer Multilevel Modelling, project aims at developing a composite multilevel platform for simulating malignant tumour development and tumour and normal tissue response to therapeutic modalities and treatment schedules.

The project aims at having an impact primarily in (a) a better understanding of the natural phenomenon of cancer at different levels of biocomplexity and, most importantly, (b) a disease treatment optimization procedure in the patient's individualized context by simulating the response to various therapeutic regimens. The predictions of the simulators to be developed will rely on the imaging, histopathological, molecular and clinical data of the patient. Fundamental biological mechanisms involved in tumour development and tumour and normal tissue treatment response such as metabolism, cell cycle, tissue mechanics, cell survival following treatment etc. will be modelled. Stem cells will be addressed in the context of both tumour and normal tissue behaviour. From a mathematical point of view, the simulators will exploit several discrete and continuous mathematics methods such as cellular automata, the generic Monte Carlo technique, finite elements, differential equations, novel dedicated algorithms etc. A study of the analogies of tumour growth with embryological development is expected to provide insights into both mechanisms.

ContraCancrum will deploy two important clinical studies for validating the models, one on lung cancer and one on gliomas. The crucial validation work will be based on comparing the multi-level therapy simulation predictions with the actual medical data (including medical images), acquired before and after therapy.

ContraCancrum aims to pave the way for translating clinically validated multilevel cancer models into clinical practice.

This deliverable identifies and characterises the anticipated user population of the ContraCancrum integrated technological platform and the uses to which it will be put. From these, it produces a broad specification for the system which will be further refined in a later deliverable.

1. Introduction

This document represents work performed within WP2. The main body provides an overview of the requirements for the various categories of user who, it is anticipated, will utilise the final ContraCancrum system. The consortium contains partners that are representative of the full range of prospective users of the system and their insights, gleaned over many years of research in their areas, have proved fundamental to this document.

The remainder of this report is as follows. Section 2 defines the objectives of this deliverable and Section 3 seeks to identify the likely users, the characteristics they have that will be most relevant to the final system, for what it is anticipated they will employ the system, when available and the shortcomings of existing systems that will be overcome by the ContraCancrum system, thus making it attractive to the various user types identified. The implications of this lead to Section 4, which considers the requirements users will have of the system in increasing detail, while Section 5 begins to envisage the possibilities that the new system will provide researchers with in the future.

There are also a number of appendices. Appendix A provides a State of the Art Report on the areas of particular activity within the ContraCancrum project to act as a reference point for future work and, within this, Section A2 requires a description of the embryological development of the brain, which is provided in Appendix B, and information on the molecular biology of gliomas, which is provided in Appendix C. Appendix D goes into detail about clinical trials and the legal and ethical constraints to be considered when undertaking them.

2. Objectives of the Deliverable

This deliverable identifies the broad needs of the ContraCancrum user population. When allied with the data map to be explored in WP3 and other tasks in WPs 2 and 3, it will form the base upon which further work will establish the requirements and specifications for the ContraCancrum integrated platform and clinical validation studies. The outcome of that investigation will be a report to be presented in PM10.

3. The Users and the Applications

3.1 Users

As ContraCancrum involves professionals from different disciplines, each of which has its own practices and speak its own "language", it is of the utmost importance that we recognise the particular characteristics of each group and ensure mutual awareness, so that each can understand the other and thus allow collective progress to the common goal.

The likely users of the ContraCancrum system can be identified as falling into three main categories:

- scientific and engineering researchers
- clinical researchers
- clinical practitioners.

The **scientific researchers**, including biologists, biophysicists, biomathematicians, biostatisticians, etc., are interested in the fundamental processes that are associated with tumour development and seek to improve an understanding of these. One would expect them to have an appreciation of the treatment regimes that clinicians adopt and the reasons that they do so. Their main goal is to gain insight into, and an understanding of, the *spatiotemporal natural phenomenon* of cancer in not just a qualitative, but also a quantitative, manner. As cancer recapitulates to some extent the entire biology, knowledge

of more generic biological phenomena such as cell cycling at several levels of biocomplexity may assist in-depth understanding of tumour behaviour and vice versa.

The **engineers**, including bioengineers, biomedical engineers, information engineers, etc., have a primary target of developing and testing integrated treatment planning systems by bringing together various simulation, image processing, visualisation and other components related to tumour growth and response to treatment.

Both of the above will have good technical proficiency, will be aware of recent technological developments and be willing to adopt a more complex technological infrastructure if it can further their aims. Most will come initially from a non-biomedical discipline and the extent of biomedical knowledge of cancer will vary, as it is typically acquired on the job to varying degrees of depth. They probably do not have first-hand experience of clinical trial methodologies and procedures though it is important that they gain an understanding about clinical trials and their regulatory requirements and guidelines.

Clinical researchers seek to improve knowledge of the effects of treatment by the retrospective evaluation of existing patients and treatments, using access to documented archives and results of parametric simulations.

Clinicians must have an excellent all-round knowledge of the different aspects of the disease and the processes (often highly complex) associated with its treatment. They have contact with patients, though such contact may be severely time constrained, and access to clinical data relating to the patients under their care. They aim to optimise treatment in the context of the individual patient. To achieve this, they have to ensure validation of the simulation systems through clinical trials. They put a high priority on patient safety and the accuracy and relevance of the treatment proposed.

While clinicians know a lot about molecular biology and clinical trial methodologies and procedures, in general, they do not understand mathematical models.

Given the interdisciplinary convergence between partners suggested above, if we consider the final system, we should also view users from another perspective – internal and external users.

Internal users are researchers of a ContraCancrum partner and are directly involved in the development of ContraCancrum demonstrators. Internal users need to be able to work with the software, integrate new models or submodels into the demonstrator, and perform the necessary tasks in order to reach ContraCancrum goals.

External users are those users who will use the outcomes from ContraCancrum for their basic research on modelling or in their clinical research. In contrast to internal users, external users will not be involved in the development process of ContraCancrum and will thus know less about the internal ContraCancrum software. External users will primarily use the ContraCancrum demonstrators without changing them. It is clear that the final ContraCancrum system will have to provide a suitable environment and background support to enable external users to employ it effectively and without excessive learning time.

3.2 The Clinical Scenario

Clinical trials are performed under very strict guidelines and regulations. The background is dealt with in Appendix D, which gives an indication of the regulations and directives that apply and the constraints on implementation of clinical trails.

Information arising from post-genomic research, and combined genetic and clinical trials on one hand, and advances from high-performance computing and informatics on the other hand is rapidly providing the medical and scientific community with new insights, answers and capabilities [1].

Up to now, the lack of a common infrastructure has prevented clinical research institutions from being able to mine and analyse disparate data sources. This inability to share the technologies and data developed by different cancer research institutions can severely hamper the research process. Similarly, the lack of a unifying architecture can prove to be a major roadblock to a researcher's ability to mine different databases. Even within a single laboratory, researchers have difficulty in integrating data from different technologies because of a lack of common standards and various technological, medico-legal and ethical issues.

As a result, it is nearly impossible to seamlessly integrate multi-level data from the molecular to the individual to the population level. It is necessary to facilitate a seamless and secure access to, and analysis of, multi-level clinico-genomic data enriched with high-performing knowledge discovery operations and services. By doing so, the development of individualised therapies in cancer will be greatly advanced. As a final goal, this approach will help to model tumour growth and therapy response realistically and reliably in the computer ('in silico' oncology). Here, 'in silico' refers to any application of computer-based technologies — algorithms, systems and data mining/analysis techniques — predicting clinical efficacy to help in clinically relevant decision-making [2].

As medicine relies on models to understand and predict the physiology and pathophysiology of biological systems, these models are verified *in vitro* and *in vivo*, but they can also be analysed theoretically with *in silico* techniques [3]. Advances in systems-biology-driven concepts in biomedicine, supported by the increasing volume of molecular data and the decreasing costs of computational power, have led to the running of many large and clinically relevant simulations [3]. If clinicians could accurately predict which treatment will fail in a patient before it is applied, it could save lives, time and resources, and might ultimately lead to more targeted, personalised therapies.

From a clinical point of view, the aim of ContraCancrum is to develop patient-specific computer simulation models of the biological activity of malignant tumours and normal tissues in order to optimise the spatiotemporal planning of various therapeutic schemes. Ultimately, the aim is to contribute to the process of effectively treating cancer and to contribute to the understanding of the disease at the molecular, cellular, and higher level(s) of complexity. Clinicians do expect that cancer growth and response to different treatments can be simulated. Such *in silico* experiments might help clinicians in future to find the best way of treating an individual patient by simulating different treatments in the computer before starting the treatment in reality. Two preconditions are of the utmost importance before one will be able to rely on *in silico* oncology models [4]:

- every *in silico* method has to be part of a clinico-genomic trial
- every prediction of an *in silico* method has to be compared with reality.

After establishing an *in silico* model, it is necessary, as a first step, to define the data required, including data from the tumour (molecular biology, pathology, imaging), from the patient (clinical data) and from the possible treatment (pharmacokinetics of drugs that will be used, the treatment schema) as well as from literature and open source databases.

To make the simulation predictions as precise and realistic as possible, it is crucial to acquire as much information from each of the different categories as practicable. However, the amount of data will be restricted by the availability of tumour material, imaging data and clinical data. Therefore, *in silico* oncology has always to be integrated into, or form part of, a clinico-genomic trial, in which data management including data security and anonymisation or pseudonymisation of data as well as tumour banking is well established. In addition, such a trial is always reviewed by an ethical committee and fulfils all other GCP criteria to gain approval by the relevant regulatory authorities [5].

The simulation prediction of each *in silico* model has always to be compared with reality (Figure 1). The feedback given by the reality has to be used to tune the *in silico* model to provide better predictions. If the treatment is to be based on predictions of an *in silico* model, such a control loop has to be part of the model and automatically executed. In this way, *in silico* experiments should be considered and established as learning systems. Only if there are no, or minimal, deviations between the prediction and reality, should the *in silico* method be allowed to be used in a clinical setting.

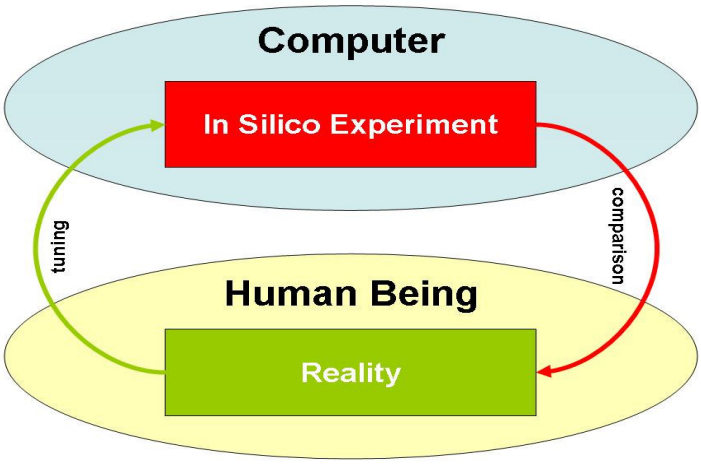

Figure 1. Comparison between *in silico* experiments and reality

The clinician has to define what is acceptable as a maximal deviation between prediction and reality in a single patient, and this definition should always be included in the biometrics part of a clinico-genomic trial protocol. For the safety of patients, a stopping rule has to be defined, if clinical decisions are based on *in silico* experiments.

For a clinician, it is important that the following main questions are addressed and answered precisely for a single patient for *in silico* experiments [4]:

1. What is the natural course of the tumour growth over time, in size and shape?
2. When and where to is the tumour metastasising?
3. Can the response of the local tumour and the metastases to a given treatment be predicted in size and shape over time?
4. What is the best treatment schedule regarding drugs, surgery, irradiation and their combination, dosage, time schedule and duration to achieve cure?
5. Is it possible to predict severe adverse events (SAE) of a treatment and to propose alternatives to avoid them without causing a deterioration in the outcome?
6. Is it possible to predict a cancer before it occurs and to recommend treatment options to prevent the occurrence or a recurrence?

In order to enter routine clinical practice as a decision-making tool, an exhaustive validation, adaptation and optimisation procedure has to take place for every *in silico* experiment undertaken by ContraCancrum. Furthermore, molecular methods of extraction of the crucial histological constitution of tumours have to be tested and integrated into the tumour-specific model. Following completion of this testing procedure, the simulation model is expected to support the clinicians' decisions concerning various candidate cancer treatment schemes and thus facilitate individualised treatment optimisation.

To use the ContraCancrum system in clinics, it is necessary that every clinical scenario in ContraCancrum fulfils the following criteria:

1. The question to be answered by the scenario is of clinical relevance.
2. The data that are needed for the scenario can be easily provided by clinicians.
3. All legal, ethical and data security requirements are fulfilled.
4. The tools provided with the scenario are easy to use by clinicians.
5. All scenarios must be validated in clinical trials before use in routine practice.

Criterion 1 can be achieved by establishing a close communication with clinicians to bridge the gab between clinical requirements and necessities and the possibilities of basic science. In this sense, ContraCancrum will play a major role in translational research. Regarding the two types of cancer tumours that are investigated in ContraCancrum such relevant questions are being asked and will be answered.

For lung cancer, the clinical scenario is well defined. A mutation analysis and sequencing of EGFR in lung cancer will be done. In a 3D model of the mutated EGF receptor, different targeted drugs will be tested in the computer to find the drug that best inhibits the receptor. In a prospective clinical trial, this drug will be given to the patient with this mutation in the EGF receptor in the experimental arm of the trial, whereas in the conventional arm of the trial, the best available therapy is given to the patient. If the outcomes of patients in the experimental arm are better than in the conventional arm, this scenario can be regarded as a test of principle and should be extrapolated to other cancers to define better and more individualised treatments for patients with cancer.

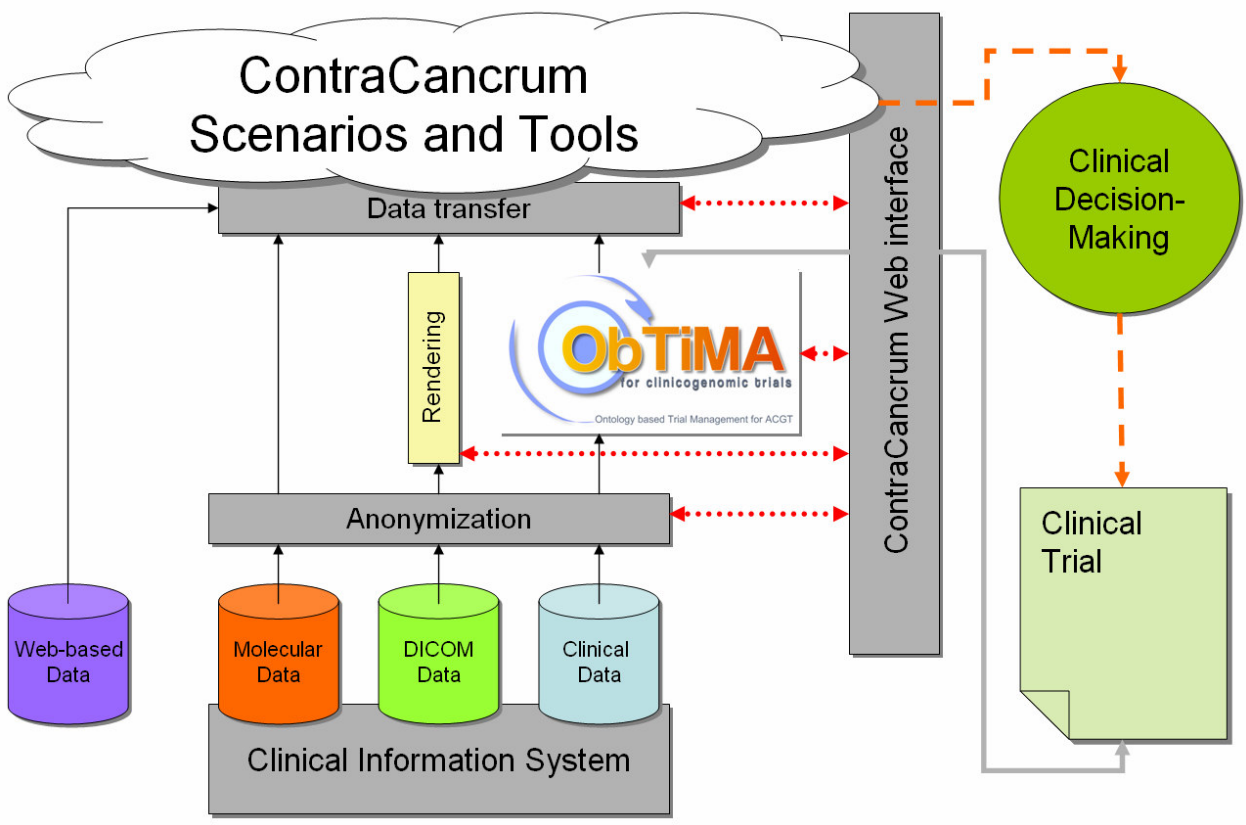

Figure 2. The use of ContraCancrum in the clinical setting

In the case of gliomas, it is necessary to further understand the complexity of these tumours, which is only possible by molecular genetic analysis. Such analysis may find common markers for reclassification of gliomas with respect to response to treatment options and outcome. Regarding the study of antigens that are associated with an immune

response, it is well known that their complexity is far less than that of gliomas. This feature may help to find relevant antigens in gliomas that may serve as new targets for treatment. Knowing relevant and glioma-specific antigens in every patient will be a big step toward an individualised therapy for brain tumours and will again be a test of principle for other cancers.

Criteria 2 to 5 are important for establishing ContraCancrum scenarios in daily clinical practice. The usability of the tools for data transfer, anonymisation or pseudonymisation of data are critical. For this purpose, ContraCancrum will evaluate ObTiMA [6], an ontology-based data-management application build by ACGT, which will support most of these requirements. Especially by establishing new clinical trials this software will be of great help. Other tools for clinical scenarios must be easily accessed via a portal system of ContraCancrum. As imaging studies are of the utmost importance in ContraCancrum, a rendering tool for tumours will be developed and tested within the project.

Figure 2 outlines the way in which the ContraCancrum system will be used by clinicians. Via a Web interface, a clinician can anonymise and transfer data to ContraCancrum. He/she will be able to use ObTiMA and a rendering tool for tumour segmentation. Results given by ContraCancrum Scenarios and tools will be accessible and will help in the clinical decision making. For validation purposes, these results will have to be validated in prospective clinical trials. For these trials ObTiMa will again be used.

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3.3 Use by Researchers

ContraCancrum is primarily targeted at multiscale cancer modelling. This will involve collaboration across different disciplines with the aim of providing new insights to assist with research, clinical practice and, possibly, relevant education. The following uses are envisaged by researchers:

- integrating the ever increasing experimental and clinical knowledge concerning cancer
- gaining insight into a plethora of biomechanisms and their interactions constituting the strongly multiscale natural phenomenon of cancer
- stimulating the emergence of new ideas regarding cancer treatment and prevention
- providing quantitative descriptions of cancer dynamics
- supporting drug discovery

- identifying treatment windows in the generic setting
- optimising the design of clinical trials
- optimising the interpretation of clinical trials
- supporting personalized cancer treatment through *in silico* experimentation (*in silico* oncology.

ContraCancrum partners have a long history of work in the area and we expect the new multiscale approach to extend and broaden their current activities, which are described below. These typify the type of work for which we envisage ContraCancrum will ultimately be used.

Partners ICCS and USAAR are involved also in the EC-funded ACGT project, within which the question of tumour response to preoperative chemotherapy is tested in children with nephroblastoma, which is the most common kidney tumour in children. An *in silico* oncology model has been established by ICCS testing this scenario in the Oncosimulator[1]. Clinical data, imaging data and data from an Antigen scenario are provided by the clinicians. Together with pharmaceutical models of enrolled cytostatic drugs the model will be tested with the real clinical response to the given drugs. A learning system as a feedback loop will optimise the Oncosimulator over time.

Following rigorous clinical validation and adaptation, the "Oncosimulator" is expected to function as a decision support tool for clinicians to optimise personalized treatment and as a training tool for medical doctors and interested patients alike.

To run the Oncosimulator for nephroblastoma, the ACGT IT-infrastructure is used, which is in compliance with all requirements and regulations for clinical trials including data security; a trial-management system, ObTiMA, is also developed within ACGT. This will be an open source tool and may be exploited within ContraCancrum, in which case, it will be necessary to develop an Ontology for lung cancer and gliomas.

Apart from the Oncosimulator, there do not appear to be any systems similar to the one proposed for ContraCancrum.

General work undertaken by ICCS includes simulation of free tumour growth, simulation of the response of tumour (*in vivo* and *in vitro*) to radiotherapy and chemotherapy and simulation of the response of normal brain tissue to radiotherapy.

Relevant work at USAAR includes the testing of a rendering tool for tumours in cooperation with FORTH; actions for developing clinical trials for gliomas and lung cancer, including ethical approval, logistics for data storage and sharing; and the measurement of autoantibodies *in sera* from patients with gliomas and running mutation analysis and sequencing of EGFR in lung cancer.

Partner UBERN has a long history of working on computer-assisted tools (navigation and planning). Current applications range from guidance for spine surgery, to dental implantology, and the latest developments include the addition of finite element analysis in these tools. The main problem being faced is providing the surgeons with tools that are easily integrated into their workflow and OR. First, the tool needs to be fast and reliable and its manipulation easy. Second, the predictions of the model need to be directly understandable by the surgeon. The "complex" numerical results need to be translated into measures used in the clinics.

[1] http://eu-acgt.org/acgt-for-you/researchers/in-silico-oncology/oncosimulator.html

In molecular modelling at partner UCL, the molecular level simulator developed and applied in ViroLab project for decision support in viral diseases treatment is being extended and generalised. It has been proved that it can obtain an accurate ranking of binding affinities of an inhibitor to wild-type and mutant HIV-1 proteases. The calculations can be completed on clinically relevant time scales with supercomputing resources.

UCL is also involved in developing agent-based dynamic models and simulations of cancer cell pathology, data mining of genomic data, application of Bayesian network methods to integrate genomic/molecular and clinical knowledge and discover cross-scale statistical dependencies. It participates in CancerGrid, a consortium of UK researchers formed to develop open standards based solutions for clinical cancer informatics (www.canergrid.org). CancerGrid has developed a grid-based network for cancer clinical trial patient entry, randomisation and follow up and methods to store and mine complex datasets, including histology, imaging, molecular and genetic markers, prognostic and predictive markers, chemotherapy and radiotherapy treatment responses and recurrence of disease. In collaboration with the National Cancer Institute (USA), the project has designed an extensive repository of common data elements and ontologies for those data types generated by cancer clinical trials (breast cancer as a specific disease type) and this repository is now integrated as an interoperable node within caBIG (cabig.nci.nih.gov).

The deliverables of the CancerGrid project provide theoretical researchers with a valuable set of data standards and, in addition, research is in progress at UCL on defined interdisciplinary workflows on how to build standardised elements for new data types and new cancer subtypes in collaboration with experimentalists and clinicians.

Industrial partner PFL-H currently has many activities including image processing. Specific activities particularly relevant to ContraCancrum include tumour segmentation, normal tissue segmentation, registration of a time series of single modality morphological images (MRI, CT), registration of a time series of multi modality data sets (PET/CT), multi-modality registration (MRI, PET, CT,…), etc.

The partners active in the image processing and visualisation tasks within ContraCancrum (WP7) provide the basic researchers working on modelling (WP4 and WP6) with services and tools to initialise the simulations. This will be supported by clinicians in order to clinically validate the image processing tools and results.

Currently, most image processing work is done on desktop workstations operated either by computer scientists in the development phase or by clinicians in the production phase.

3.4 Current shortcomings

In designing the ContraCancrum system, we should take account of shortcomings in current approaches, whether inherent or induced by external circumstances, and thus try to ameliorate them as much as possible in the final implementation.

Cancer informatics platforms may allow data mining of multi-scale patient data. In the CancerGrid system, for example, modelling the response to epidermal growth factor receptor (EGFR) inhibitors for predicting cancer cell fate (cell death or proliferation) requires information on molecular mechanisms, cell network dynamics, mode of action of therapeutic EGFR inhibitors and clinical parameters for dynamic simulation.

However, they do not support dynamic predictive simulation of individual treatment response, so a new type of system is needed that will integrate multi-scale clinical data captured from individual patients with detailed knowledge about biological processes in cancer cells and tumours from fundamental research in dynamic simulations – ContraCancrum is designed to fill this gap.

There are significant problems with data. Data are not standardised and often lack sufficient metadata. They are also generally scattered, so access is very laborious. There is a paucity of adequate multiscale data to be used for adaptation and validation purposes, which is exacerbated by the lack of standardisation, and of other molecular biological data for the Oncosimulator.

In addition there is a lack of experimentally and clinically validated knowledge of the effect of certain molecular mechanisms on tumour response to radio- and chemo- therapeutic treatment and hence this is not formalised in computational models.

It is clear that ContraCancrum cannot, by itself, resolve such a large issue but it will ensure that any data collected will conform closely to standards and will have suitable metadata. Further, by discussion within the VPH community it will try to ensure compatibility across other similar projects.

In relation to imaging, even if a standard is prescribed, patients might present at clinic with pre-existing imaging studies that do not observe these standards. To repeat such imaging is not possible because of the associated cost, so it will never be possible, in a project involving real clinical situations, to ensure that all imaging is in standardised form.

Once images are available, tumour and normal tissue segmentation requires a great deal of time-consuming manual interaction with the data sets, and the registration results are often not clinically verified.

Further, a suitable data transfer and workflow mechanism for multi-site collaboration has not yet been established – the current usage paradigm is that of single user performing the full list of image processing tasks with tools that are installed on a single workstation on his/her desktop.

Other shortcomings include the lack of an ontology for glioma and lung cancer, which will be necessary if a system such as ObTiMA is to be used, the deficiencies in rendering tools that reduce their usability by clinicians and the time needed to setup clinical trials, with regard to the requirements and regulations for clinical trials within Europe.

4. Users' Requirements

4.1 Overview

The ContraCancrum system will be characterised by well designed multiscale data collection, the exploitation of a large number of data sets concerning the two cancer types addressed (i.e. gliomas and lung cancer) and integration of the biochemical and detailed biomechanical levels. It will be capable of interconnecting with other systems and of utilising distributed resources effectively.

It will provide fast and reliable patient-specific tumour prediction, patient follow-up, multimodality morphometric analysis of tumoural lesions, validation and improved communication of the results to the clinician.

The ContraCancrum image processing demonstrator will allow the creation of the input necessary for the simulations in WPs 4 and 6 and will include:

- segmentation of tumours and tumour subvolumes
- segmentation of healthy tissue, including as a minimum
  - Brain: ventricles and other fluid filled space, grey matter, white matter, skull
  - Lung: airways, vessels
- registration

- time series of images, multimodal registration, globally rigid registration in the brain, locally rigid registration in the lung.

It will allow image-processing scientists to work cooperatively on datasets and perform the required processing steps at different sites on different image processing platforms and clinicians to view, verify and approve the results of image processing operations that were performed at other sites. It will reduce the workload to clinicians as well as image processing scientists by partly or fully automating the image processing operations.

Successful application of the molecular level simulator relies on initial biomolecular structures and force field parameters. However, crystal structures of epidermal growth factor receptor (EGFR) - inhibitor complexes are not always available. Structural modelling and/or docking methods may be needed for preparation of initial structures before use of the molecular level simulator.

The existence of the ContraCancrum system will open new possibilities such as deepening the biochemical understanding of the interaction of specific drugs with specific proteins. It will allow refined mechanical modelling of the tumour/adjacent-normal-tissue system and the eventual identification of the analogies of glioma development with the embryological development of normal brain.

From the perspective of theoretical researchers, enhanced possibilities will include the effective interdisciplinary communication and modelling of physiological processes and clinical scenarios driven by diverse data types, which is vitally dependent upon transparency of systems and interface design, and deployment of standardised vocabularies, ontologies, common data elements and metadata descriptions. Workflow models will be used to define common data elements relevant to experimental and clinical measurements of the DNA damage response, which will drive the ContraCancrum agent-based models under WP5.

4.2 Constraints

We recognise that many factors may inhibit the success of any deployed system and that circumstances may often affect its performance. By identifying constraints early in the development process, it is often possible to mitigate their effect or circumvent them completely.

One of the most significant is time that is needed for developing a clinical trial according to the requirements by regulatory bodies; this may become a problem if early results suggest items that were not originally included and which time pressures may then prevent from being agreed and undertaken.

Legal restrictions on the distribution of data due to data privacy regulations may create unexpected problems.

Clearly straightforward issues such as ensuring a suitable volume and accuracy of the data collected, variations in the standards of the data collected, etc., have to be addressed directly within the processes of the project.

Many of the issues may emerge only when the system is deployed and it will not be possible to predict their exact form in advance. For these, it is important to retain an awareness of the possibility that they may occur.

For the physical system, the communication and interaction amongst heterogeneous computer systems based in different locations, and thus the synchronisation of complex integrated simulation processes or interactive input into the simulator, may generate specific localised difficulties. If the system becomes highly complex, and the volume of data to be transferred and subsequently processed expands, the overall computation times may

become excessively large. Security practices at different locations may require particular attention.

From the perspective of the systems biology modeller, crucial gaps of knowledge in cell network function may be revealed in the course of model building. Contingency plans for this situation would shift the level of abstraction to larger functional modules within the cell and would apply Boolean network modelling, which can deal with missing mechanistic knowledge in a large range of circumstances.

In interaction between components of the system, a lack of sufficient data from molecular biology to describe gliomas or lung cancer precisely and accurate may present difficulties. The autoantibodies in gliomas or the EGFR mutation and sequencing do not provide sufficient data to describe the individual tumour of a patient in an order that is needed for a tumour model in the oncosimulator. In this case, the question that should be answered by the oncosimulator should be precisely addressing the provided data, e.g. "Is the mutation analysis of EGFR sufficient to predict the response to the best simulated targeted therapy?"

Particular issues that should be considered carefully in the system design include the usability of the system for clinicians, including the tools needed to provide the data, and tools running in the background that are needed for data security (such as anonymisation, etc.)

The input-output data feeds between higher-level model components (especially those concerning data captured *in vivo* from the patient) and the predictive agent-based simulation of the likelihood of cell kill have to be very clearly defined from the start

4.3 Desirable Features

Features identified as desirable include reliability, modularity and extensibility. These imply that the system is scientifically sound, technologically advanced and clinically validated, that it has a modular nature so that improved modules can replace previous versions easily during the evolution of the system, and that it can be extended to other tumour types and treatment strategies with minimal changes in its scientific and technological core.

User friendliness is particularly important in relation to use by clinicians with regard to both the form of interaction in which they have to engage and the way in which information is presented to them, which should be timely and meaningful in the clinical context.

There should be tools for data security, data sharing, the management of clinical trials (such as ObTiMA) and visualisation and a Web-based workflow management system.

Data formats for the exchange of data should be fully and clearly defined.

The system should be readily accessible from any location and, likewise, access to the relevant data should be straightforward. The system should produce results in acceptable times, where "acceptable" may have a different scale within the clinic than within a research laboratory.

4.4 Detailed Requirements

From the previous discussion we have arrived at a set of detailed requirements from the users. The final system should demonstrate the ability to:

- address the required data, by legislative and ethical requirements, anonymisation and privacy requirements;
- pseudo-anonymise patient data and store them in dedicated ContraCancrum databases, together with all the relevant patient information (clinical, histopathology, genetic information, etc.);

- load data from different technological platforms, usually involving DICOM servers, by utilising Grid/web-services
- apply various medical imaging analysis tools mainly for registering data and outlining (segmenting) the Region of Interest (ROI) in all patient 3D Imaging data and storing the results back in the central database of the project;
- enable the clinician to score different segmentation results and 'accept'/'reject'/'correct' the cancer delineation for each case;
- apply biomechanics concepts in all patient 3D Imaging data and storing the results back in the central database of the project;
- simulate cancer growth and response to therapy at the cellular level (the segmented ROI is a basic input);
- exploit simulations at the sub-cellular level;
- use high-quality visualisation tools (possibly incorporated as web services), so that the clinician can visualise both the original segmented tumour and the simulation result(s) (both stored in the central database) and 'score' each simulation – this is, in essence, the 'validation framework' for the project;
- demonstrate "elementary workflows" for the glioblastoma and lung cancer clinical scenarios.

Surgeons and researchers should be able to work from any place, submit data easily and visualise simulation results. The different components of the simulator should be accessible from a central location and the user should be able to start simulation processes remotely, which implies Web-based submission of data and visualisation of the results. It is likely that simulations will be performed on remote (super-)computers to improve computation times – if this implies grid computing, access should be in a transparent way for the end user – but user interaction should be possible, for example, the clinician should be able to check the tumour segmentation and eventually suggest corrections.

Access to the system should be managed by roles and rights. Full descriptions of the available tools and training support mechanisms for their use should be provided and the data available should be easily searched for the most appropriate data for the user's needs.

Upload of new modules should be straightforward.

The protocols used for relevant clinical trials should be available for the scientific researchers.

As a final point, it is felt important that the outcomes from the project remain usable long after the project termination date. In particular, the data-collection protocols should ensure that the data collected during the project remains available into the future.

5. Future Vision and Implications

We anticipate that the final system and the associated access to distributed resources, tools and data will make a significant impact on the work of the users and provide them with many further opportunities to advance their work. The final multi-scale system will be a powerful integration technology linking molecular-level and higher-level processes in tumours that will be incrementally extensible through the integration of further models representing diverse physiological processes and treatment repose modalities in tumours.

The system will contribute to the emergence of an increased level of interdisciplinary investigations and new communities of practice. It has the potential to drive culture change in cancer research and clinical investigations in the direction of personalised medicine,

provided that due attention is paid to the institutional, social, cognitive and psychological challenges of change in research practice and interdisciplinarity.

For clinicians, the additional knowledge with respect to system biology will provide an increased understanding of the disease and its treatment and thus help to optimise therapy. It will assist with progress towards patient-specific treatment and will provide better treatment accuracy and predictability, improved patient safety and opportunities for the evaluation of different treatment strategies.

Experienced gained should assist with future clinical trial simulation, design and interpretation. The successful cooperation of clinicians, molecular biologists, IT professionals and other basic researchers will make it easier to bring multidisciplinary knowledge into patient care in the future.

As a by-product, clinicians will have to learn more about IT and begin to appreciate the possibilities opened up by the availability of oncosimulators and similar technology. This may also provide momentum towards changing curricula in medical schools and developing a more open view towards further introductions of IT in the clinic.

Researchers will acquire better knowledge of the mechanisms of tumour growth and will gain insights into improved treatment strategies. For example, through model integration, systems biology modellers/theoretical researchers will be able to translate experimental and clinical knowledge into increasingly complex multi-scale models for clinical use. Theoretical researchers will be able to access clinical knowledge formalised in the system and to integrate this with their molecular-/network-/cell-level models.

Enhanced access to high performance computers may provide a new perspective on the possibilities for further research and may lead to a greater level of integration of computer-assisted decision making in future products.

Appendix A. State of the Art Report

A1. The integrated ContraCancrum scientific and technological platform

A1.1 Introduction

As noted in the Description of Work, ContraCancrum intends working on a broad front of activities, using the strong multidisciplinary character of the ContraCancrum consortium to achieve advances on the state of the art in a number of separate areas as well as driving progress by the development of a consolidated approach across the various strands of activity, thus reducing disparity and providing enhanced opportunities for cross-fertilisation.

ContraCancrum will advance cancer modelling research by integrating biochemical, molecular, cellular, tissue, organ and physiological system modelling concepts with medical image analysis and tissue biomechanics into a single technological entity that will simulate therapy outcome based on the individual patient's data. This will provide a powerful weapon in the understanding of, and fight against, cancer.

The ContraCancrum project will be driven by real clinical needs and will utilise real clinical data in order to demonstrate the added value of modelling and pave the way for its future use on the clinical setting. In order to be as realistic as possible and at same time provide a considerably generic computational platform, both tumour and normal tissue behaviour modelling will be approached through a number of paradigmal cases. Two dedicated clinical studies will run within the project: one in lung cancer and one in glioma. The studies will provide in total about 200 cases per year to be used for the optimization and validation of the ContraCancrum integrated simulator.

To go beyond the state of the art, we shall consider as much information as possible stemming from different levels of biocomplexity for each individual patient. For this reason the project workplan will integrate molecular models of cancer with models of the cellular and higher biocomplexity levels thus integrating different scales. Additionally, the models will be individualised by modelling tissue biomechanics and extracting anatomical and functional information using sophisticated medical image analysis techniques. The "summarize and jump" strategy, aiming at a pragmatic biocomplexity "level jumping" and integration[2] will serve as the core philosophy for the multilevel integration of biological data and mechanisms involved in ContraCancrum cancer modelling:

The following aspects are specifically addressed in the project:

i. the integrated ContraCancrum scientific and technological platform
ii. clinical aspects of gliomas and brain embryology
iii. clinical aspects of lung cancer
iv. simulation of tumour and normal tissue behaviour at the cellular and higher levels of biocomplexity
v. biochemical modelling of tumour response to treatment
vi. molecular modelling of tumour response to treatment
vii. biomechanical modelling of tumour and adjacent normal tissue
viii. image processing techniques in oncology.

Each of these topics is each treated within its own section below, and numbered as indicated above. The references are presented within the associated section and are numbered accordingly.

[2] G. Stamatakos 2006, NCI CViT Ask the Expert Forum, password-controlled website https://www.cvit.org/node/128

A1.2. State of the art

To the best of our knowledge, tumour growth models developed up to now (see section A4), have been confined within the theoretical research environment. One exception is the "Oncosimulator" of the EC-funded ACGT project, which is still under development. This is the first tumour-growth and treatment-response simulation model to be explicitly positioned within the clinical environment – it has been designed according to actual clinical needs and is being integrated into clinical trial technologies and adapted to running clinicogenomic trials. It is also being optimised and validated by employing trial-generated data. Three ContraCancrum partners are playing leading roles in the development, validation/translation and technological support of the ACGT "Oncosimulator", so there will continue to be a good information flow between the two projects.

A1.3. Expected results

As ContraCancrum will explicitly integrate an impressively larger number of biocomplexity levels, spanning the quantum chemical level to the physiological system level, will study different cancer types (gliomas and lung cancer) and will also address pathogenetic mechanisms (e.g. glioma emergence vs. embryo development), it may be considered as the precursor of a "second generation" of oncosimulators.

Further, the direct and orchestrated involvement of six University Hospital Departments (USAAR) will provide 100 clinical trial cases for gliomas and 100 clinical trial cases for lung cancer per year for the optimisation and validation of ContraCancrum and this is expected to bring a further significant advance to *in silico* oncology. Such an anticipated outcome would be another European first.

The development of an advanced technological environment for the functioning of the integrated simulator that will provide access to grid computational resources, web-based remote access, access to anonymisation services etc. will render ContraCancrum efficient, reliable, user friendly and compatible with legal and ethical constraints. The resulting pre-commercial version will have considerable potential for future commercialisation.

A2.Clinical aspects of gliomas and brain embryology

A2.1. State of the art

Human gliomas are the most common primary central nervous system neoplasms. The mechanisms that control the insidiously invasive nature of malignant gliomas are poorly understood. Glioma tumour cell growth (cell division and differentiation), vascularisation and invasiveness (cell migration) are the most important steps in the development of gliomas. Embryology may provide insights into neoplastic development in general.

Background information on the embryological development of the brain which may shed more light on the development of gliomas is provided in Appendix B.

Improving the understanding of the molecular mechanisms in brain development, especially cell division and differentiation, migration, vascularisation and apoptosis will make a significant difference to the understanding of brain tumours. Regarding molecular pathogenesis, the main focus has to be put on cell-cell interactions, cell division and differentiation, migration and vascularisation.

It appears that morphogens play unexpected roles in the development and degeneration of the central nervous system. Homeobox genes and signalling factors both play a major role in this setting. In early development, embryos transform from a simple group of cells into an organism of complex shape. This occurs through a precise re-arrangement and re-positioning

of cells and tissues. Tracking how this process of shaping occurs is thus expected to help with the understanding what goes wrong in cancer. Many steps present in the normal shaping of embryos reappear in an uncontrolled fashion in malignant tumours. While surgery, radiation therapy and chemotherapy have roles to play in the treatment of patients with gliomas, these therapies are self-limited because of the intrinsic resistance of glioma cells to therapy and the diffusely infiltrating nature of the lesions. It is now known that malignant gliomas arise from a number of well-characterised genetic alterations, activations of oncogenes and inactivation of tumour suppressor genes.

The recognition of the putative brain-tumour stem cell, the tumour-initiating cell in brain cancer, provides an enticing target through which we could eliminate the source of the brain tumour with increased efficacy and less toxicity to normal tissues. Appendix C provides several tables from Bansal et al. [2-1]: Table C1 shows proto-oncogenes involved in gliomas; Table C2 shows several common tumour suppressor genes involved in gliomas; Table C3 presents some common genetic aberrations in familial astrocytic tumours.

Bansal et al. [2-1] described the glioma signalling pathway in a review on the molecular biology of human gliomas. It has long been accepted that tumours are dependent on angiogenesis, the directional sprouting of new blood vessels from existing vessels within the tumour [2-2]. During embryogenesis, the normal physiologic creation of new vasculature is termed vasculogenesis, whereas the budding of capillaries from existing blood vessels is called angiogenesis [2-3]. Tumours must secrete diffusible chemicals that stimulate endothelial cells via a paracrine effect and leads principal cell type contributes to angiogenesis. The process of angiogenesis has been quantified by measuring microvessel density in tumour specimens [2-4].

Recently there is increasing evidence for antibody response against proteins expressed in human tumours. Tumour cells are characterized by altered pathways and/or altered proteins that can be recognized by the human immune system. Antibodies that can be detected in patients' blood offer a unique possibility to monitor tumour development without being dependent on tumour biopsies. Most recently, multiple antibodies were found for several human tumours indicating a complex humoral immune response in tumour patients. In 2005, complex seroreactivity patterns were reported for human tumours including one study on prostate cancer and another study by partner USAAR on meningioma, which is a generally benign human cranial tumour. This reported not only the first evidence for specific seroreactivity pattern in benign tumours but also evidence for reactivity pattern specific for tumour subtypes [2-5].

The heterogeneity of gliomas is well known. This is due to the complexity in histology as well as in molecular genetic findings. From a clinical point of view, the current treatment of gliomas is mainly based on histology and the WHO grading of the tumours. Very few molecular findings are known that are associated with response to treatment and outcome. The chemotherapy drug temozolomide, first tested in recurrent glioblastoma and recently approved for use in those tumours, increases the life span in some patients by a number of months. A few participants in early clinical testing of this drug are still alive some years beyond the original expectation. We do know that resistance to Temozolomide occurs in many patients who have a specific DNA repair gene (0-6-methylguanine-DNA methyltransferase or MGMT) that remains silent during treatment. Temozolomide does not help patients with normal MGMT gene.

To further understand the complexity of gliomas, it is necessary to perform molecular genetic analysis in these tumours. Such analysis may find common markers for reclassification of gliomas in respect to response to treatment options and outcome. Regarding the study of antigens that are associated with an immune response, it is well known that their complexity is far less than that of gliomas. This feature may help to find

relevant antigens in gliomas that may serve as new targets for treatment. Knowing relevant and glioma-specific antigens in every patient will represent a major step towards individualised therapy for brain tumours.

In particular, partner USAAR recently showed that autoantibody profiles allow an accurate discrimination not only between glioma patient sera and normal sera but also between glioma sera, sera of patients with other intracranial tumours (meningioma, pituitary adenoma, acoustic neurinoma, metastatic tumour) and sera of patients with non-tumour brain pathologies (multiple sclerosis, chronic inflammatory polyradiculoneuropathy). It remains unclear if different glioma subtypes can be distinguished using the autoantibody pattern of patients with different gliomas – this will be evaluated during the project.

Further information on the state of the art on the glioma pathology, molecular biology, treatment and the humoral immune response against glioma expressed antigens can be found in references 2-6 to 2-47.

A2.2. Expected results

This project aims to pioneer the field of serum-based diagnosis in human glioma. It will be the first study to identify a large number of antigens that are associated with an immune response in patients with glioma. This information will be used to differentiate tumour sera from normal sera and subsequently to differentiate subtypes of glioma based on serum analysis only. Such blood-based analysis will greatly improve the information about the tumour prior to surgery.

Currently, this information is provided only by imaging technology, which cannot differentiate between different glioma subtypes. Our approach will offer, for the first time, an avenue to gain such insight with immunogenic antigens which are utilised as reporters for glioma. Serum-based molecular data in conjunction with imaging and clinical data will be used to simulate the treatment response in an *in silico* experiment. Comparison of the simulation predictions with clinical reality (treatment outcome data) will be used in order to optimise and validate the ContraCancrum integrated simulator that will be developed within the framework of the project.

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A3. Clinical aspects of lung cancer

A3.1. State of the art

Lung cancer is the most common cause of cancer deaths among both men and women in western countries. Small-cell lung carcinoma is distinct from other kinds of lung cancer as metastases are already present at the time of discovery. Non-small-cell lung carcinoma (NSCLC) accounts for 80% of all lung cancers and is usually subdivided into squamous cell carcinomas (35%), adenocarcinomas (27%) and large-cell carcinomas (10%) [3-1].

Clinically evident lung cancers have genetic and epigenetic changes in tumour suppressor genes and/or oncogenes, resulting in selection of clonal cells with uncontrolled growth capacities [3-2 to 3-4]. Basic research into the function of these molecules – how and when they play their role – should help to fight against lung and other cancers and give clues to find appropriate therapies.

Tumour suppressor genes, in their unmutated form, play critical roles in controlling the normal cell growth. Generally, they inhibit tumorigenic processes and could be involved in DNA repair. TP53 mutations are amongst the most frequent abnormalities occurring in 80-100 % of SCLC and 50-80 % of NSCLC. Overexpressed or constitutive active oncogenes are important targets for anticancer therapy. Most promising are inhibitors of receptor tyrosine kinases, because they play critical roles in transformation, proliferation, motility, and survival of cancerous cells.

Receptor tyrosine kinases associated with lung cancer are the human epidermal growth factor receptor (HER) family including epidermal growth factor receptor (EGFR), insulin-like growth factor receptor (IGFR), vascular endothelial growth factor receptor (VEGFR), c-Kit, c-Met, and the ephrin family of receptor tyrosine kinases [3-5]. The most investigated receptor tyrosine kinase EGFR is overexpressed in at least 80 % NSCLC. But there are also mutant forms of EGFR, which result in ligand-independent constitutive active receptors. Activation of EGFR, leads to the activation of several pathways that results in cell proliferation, differentiation, migration, adhesion, and protection from cell death. EGFR gene mutations can alter the sensitivity of lung cancers to drugs that inhibit EGFR kinase activity.

Mutations in the ATP-binding site, which is also the binding site for gefitinib (Iressa), result in increased responsiveness to that anticancer drug [3-6]. In contrast, the mutation of threonine 766 is responsible for resistance to some tyrosine kinase inhibitors [3-7]. Under normal conditions the EGFR signalling pathway is as follows: When a ligand binds to the receptor, homo- or heterodimerization occurs. Dimerization is followed by receptor autophosphorylation on tyrosine residues, which mainly occurs by one receptor molecule phosphorylating the other in the dimer [3-8, 3-9]. This event triggers a series of intracellular pathways.

Further information on the state of the art on the lung cancer pathology, molecular biology and treatment can be found in references [3-10 to 3-16]

A3.2. Expected results

Within the framework of ContraCancrum, analysis of the tyrosine kinases in the tumour material of patients with lung cancer will be performed. Mutation analysis and sequencing of the kinases will also take place. From the sequences, a 3D model will be developed in the computer and known targeted drugs will be screened to find those that fit best to the patient's individual pattern of tyrosine kinases. This individual pattern will be used in case of a relapse to treat the patient with the best drugs available.

For this purpose, a clinical study-scenario has to be set up using the ACGT environment. This scenario will be set up in the same way as the glioma scenario. The response to treatment will be measured clinically and compared to the predictions of the ContraCancrum integrated simulator. Thus an *in silico* trial will run in parallel with the actual clinical study.

The ContraCancrum integrated simulator will be optimised by exploiting the feedback generated by the comparison of virtuality with reality. The glioma and the lung cancer scenarios will both be the first worldwide to address cancer biocomplexity levels spanning from the quantum chemistry scale up to the macroscopic imageable tumour scale and further up to the physiological system scale, both in clinical reality and *in silico*. The physiological system scale has to also be considered in detail in the multilevel simulations since drugs continuously undergo pharmacokinetic interactions with the human body before, during and after the expression of their pharmacodynamic effect.

Partner USAAR has long experience and proven expertise in designing and running such oncological clinical trials/studies/scenarios.

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A4. Simulation of tumour and treatment-affected normal tissue behaviour at the cellular and higher levels of biocomplexity

A4.1. State of the art

Over recent decades, substantial progress has been made regarding the development of mathematical and computational models simulating various aspects of tumour behaviour. The majority of the models adopt the "bottom-up" approach, i.e. they address biocomplexity by starting from its lower levels (molecular or cellular) and then try to reach higher and higher levels. Such models refer to the *in vitro* development of tumour spheroids or the *non-imageable* preangiogenetic development of tumourlets within the organism by focusing on several mechanisms or combinations of mechanisms [4-1 to 4-6]. Angiogenesis in tumours has been addressed by several investigators [4-7 to 4-9]. Invasion has also been the subject of a number of theoretical investigations primarily addressing glioma invasion [4-10, 4-11]. Concerning the modelling of *imageable* large *in vivo* tumours, most models tend to focus on the growth aspect by trying to predict the shape of the tumour as a function of time [4-12 to

4-14]. Certain models have also been developed in order to simulate the response of numbers of non-mutually-interacting tumour cells or small non-imageable tumourlets to therapeutic interventions [4-15 to 4-31]. In the clinical context, such models are potentially useful in order to give a rough estimate of the relative effectiveness of various candidate treatments on non-imageable tumourlets. This is the case when only some population-based statistical knowledge is available concerning the invasiveness and/or diffusiveness of a specific tumour type instead of exact imaging data (i.e. non-imageable glioblastoma micrometastases).

However, as from the clinical point of view, it is the prediction of the response of an *already grown* and *imageable* tumour to several candidate therapeutic schemes that is of the primary importance and, at the same time, this is the case where models can be strictly validated in a *quantitative* (imaging based) manner in the *clinical setting*.

Partner ICCS has focused its research work on the latter aspect of *in silico* oncology. To do so, the group developed a "top-down" simulation approach for cancer treatment which starts from the macroscopic imaging data and subsequently integrates information stemming from lower and lower biocomplexity levels. In collaboration with experimentalists, radiobiologists and clinicians, it has produced a number of treatment simulation models addressing both tumour and treatment-affected normal tissue behaviour that are based on the individual data of the patient (imaging, histopathological, molecular and clinical) [4-32 to 4-44]. Figure A4-1 shows a typical three-dimensional rendering of predictions from these models.

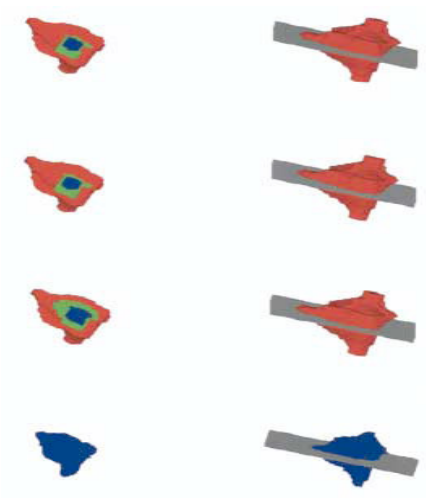

Figure A4-1. Irradiation response simulation of an imageable glioblastoma tumour according to the standard fractionation scheme (2 Gy once a day, 5 days per week, 60 Gy in total).
Left panel: 3-D sections of the tumour shown in the right panel. *Top row*: before the beginning of irradiation. *Second row*: one fictitious day after the beginning of irradiation. *Third row*: two fictitious days after the beginning of irradiation. *Bottom row*: three fictitious days after the beginning of irradiation. Colour code: *red*: proliferating cell layer; *green*: dormant cell layer (G); *blue*: dead cell layer. The colouring criterion "99.8%" used to visualise the predictions has been defined as follows. "For a geometrical cell of the discretising mesh, if the percentage of dead cells is lower than 99.8% then if percentage of proliferating cells > percentage of G cells, then paint the geometrical cell red (proliferating cell layer), else paint the geometrical cell green (G cell layer), else paint the geometrical cell blue (dead cell layer)." The values of certain parameters (e.g., cell loss) have been deliberately exaggerated in order to facilitate the demonstration of the ability of the model to simulate the shrinkage effect. Adapted from [4-32].

An initial clinical trial-based retrospective validation of some models has already been achieved [4-35 to 4-45], while within the EC funded ACGT project, new *in silico* oncology "top-down" models have been developed and tested by exploiting actual running clinicogenomic trials [4-46 to 4-47]. The primary target of this approach is to provide the clinician or the researcher with a system of *simulators* in order to perform *in silico* experiments regarding the likely outcome of several candidate therapeutic schemes/schedules for any given individual patient.

The experiments refer to the treatment response behaviour of tumours and, to a lesser degree of detail, of normal tissue. The simulators will act as dynamic integrators of the multilevel data and mechanisms corresponding to the spatiotemporal natural phenomenon of cancer as exhibited in the individual patient. Subsequently, following a rigorous clinical optimization and validation, the clinician is expected to be able to decide on the optimal treatment scheme and/or schedule to be administered to the particular patient based on the predictions of the simulators and his or her own formal medical education, experience and logic.

A4.2. Expected results

The cell and higher biocomplexity level simulators of the project will be primarily based on the "top-down" simulation approach described above which will be applied for the first time to the simulation of treatment response of lung cancer. The same approach will also be considerably refined and adapted for the case of gliomas. Special emphasis will be put on stem and limited mitotic potential tumour cells.

In this way, multilevel information will be being extracted and used in a realistic level of detail by always taking into account that the whole endeavour is to be confined within the clinical setting. However, the "bottom-up" approach will also be adopted in simulating non-imageable tumour growth, especially the process of glioblastoma local invasion. It is noted that in parallel with the tumour-response models, rather simple experimentally and clinically based toxicology models, in combination with discrete simulation of the replenishment of normal tissue stem, transit and differentiated cells, will provide safety limits beyond which any candidate treatment scheme would be clinically unacceptable regardless of the predicted outcome of its tumour control. The multilevel clinical testing, optimisation and validation processes to be performed mainly within the framework of WP8 and WP9 will constitute *per se* another novelty of the project in the context of gliomas and lung cancer.

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A5. Biochemical modelling of tumour response to treatment: patient-specific chemotherapy drug targeting

A5.1. State of the art

Targeted therapy refers to a new generation of anticancer drugs that are directed against cancer-specific molecules and signalling pathways. They are designed to interfere with a specific molecular target, usually a protein with a critical role in tumour growth. Tyrosine kinases are one such especially important target because they play an important role in the transduction of extracellular signals to the cytoplasm. Molecular dynamics simulations on a computational grid can be used to study the affinity of inhibiting tyrosine kinases on different (patient-specific) receptors, thus providing more effective design of chemotherapeutic strategies for patients.

A recent FP6 e-Health project called VIROLAB is such a patient-specific virtual laboratory which functions as a rule-based decision support system for the treatment of infectious disease [5-1].

The receptor tyrosine kinases (RTKs) are membrane-spanning cell surface proteins (Figure A5-1). They are characterized by immunoglobulin-like extracellular domains that have a ligand-binding site, a transmembrane segment, and an intracellular domain that includes tyrosine kinase (TK) catalytic sites. Blockade of ligands binding and inhibition of activated receptors are frequently used methods to control tumour growth in numerous preclinical models. Tyrosine kinase inhibitors (TKIs) interfere with specific cell signalling pathways and thus allow target-specific therapy for selected malignancies. Some TKIs have been approved for use in cancer therapy, and several others are in various stages of clinical trials.

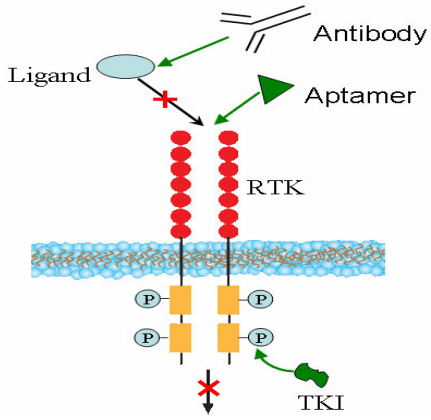

Figure A5-1. Receptor tyrosine kinase function

More than half of the RTKs have been found to be overexpressed or mutated in tumour cells. These mutations allow cancer cells to develop drug resistance and escape therapy. Clinical studies show a strong correlation between the presence of mutations and patient response to tyrosine kinase inhibitors [5-2]. Mutations usually occur in the receptors' kinase catalytic domain and are clustered around the ATP-binding pocket of the enzyme. They associate to the development of drug resistance. The resistance is introduced by preventing or weakening the inhibitor-receptor binding. Recently, mutations in the epidermal growth factor receptor (EGFR) have been identified in lung cancer [5-3]. Patients who express these mutations have shown some differences in the clinical response. Mutation in the RTKs is a contributing factor to the eventual failure of treatment. A complete description of mutations on catalytic efficiency must therefore be crucial in the clinical interpretation.

Further information on the state of the art on the biochemical modelling of tumour response to treatment can be found in references [5-4 to 5-9]

A5.2. Expected results

To facilitate the strategic design of safer, more effective and durable therapies, it is important to explore the molecular mechanisms of drugs with their targets. We will provide, through molecular dynamic simulations, molecular data on the binding affinities of inhibitors to RTKs. The simulations will significantly improve the understanding of the mechanisms that regulate signal transduction.

A5.3. References

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A6. Molecular Modelling of Tumour Response to Treatment: Molecular Interdependence Networks for Cell -Survival Probabilities

A6.1. State of the art

The therapeutic goal of cytotoxic treatments (radio- and chemo-therapies) is to trigger apoptosis in tumour cells by inducing DNA damage. However, treatment is still severely challenged by the complex system properties of tumour cells. It is now established that tumour cells can acquire resistance to cytotoxic therapies by developing a DNA *damage-tolerant* phenotype, whereby even persistent DNA damage can become uncoupled from apoptosis through abnormal signal processing and deregulated feedback control in the DNA damage-response (DDR) and apoptotic pathways.

Deregulation of these pathways is driven by multiple mutations and altered expression of many proteins. Consequently, prediction of cell survival probabilities needs to take into account the complexities of multiple molecular determinants of resistance at the level of signal-processing networks in the cancer cells. Analytical methods are here limited in usefulness.

In contrast, agent-based modelling (ABM) is well suited to dynamic biological systems. ABM is perhaps the most commonly used technique in complex systems science [6-1] since it

allows the simulation of emergent 'macro-level' behaviours from lower-level interactions between individuals or agents. For example, Abbott *et al.* [6-2] implemented the cellular phenotypic changes of the 'hallmarks of cancer' in an agent-based simulation to test the hypotheses proposed by Hanahan and Weinberg [6-3]. However, ABM is still underutilised for the modelling of cell networks and cell-fate decisions, such as cancer-cell death in response to cytotoxic treatment.

Although the analytical approach, in which several differential equations are used to represent a dynamic system, is extensively used in biology, it has been widely noted that such an approach has its limitations in modelling biological systems in which there are many interactions between the individual components, especially when these interactions are local, such as those in signalling networks. Since the differential equation approach assumes that the current state of the system is a consequence of the previous *global* state of the system, it does not capture the interactions between individuals in the system. There are many different clinical strategies for optimal therapy, so the optimisation problem has become very complex, consisting of many sub-branches. Cell-survival decisions in response to cytotoxic treatment (radio- and chemo-therapies) can be conceptualised in terms of the emergent properties of the cell mediated in large part by the DDR network. Complexity science still lacks a formal description of emergent properties and behaviours in terms of lower-level agents and behaviours, and as a consequence, there are few techniques in the literature for empirically investigating specific emergent properties.

Partner UCL has recently developed a method for analysing emergent behaviours in multi-agent simulations using complex events, which are compositions of events generated from component-level rule executions. Complex events are composed of interrelated events and can be defined at any level of spatio-temporal abstraction (equal to, or above, the lowest level of abstraction given by the model) [6-4]. The formalism is related directly to the rules driving the component behaviour so that all higher-level behaviours can ultimately be decomposed into rule executions; this is particularly important where intervention at the component-rule level is needed (i. e., in modelling the impact of different treatment regimes on cell-fate decisions at the molecular-network level).

Further information on the state of the art on the biochemical modelling of tumour response to treatment can be found in references [6-5 to 6-9]

A6.2. Expected results

The complex event formalisms of partner UCL [6-4, 6-9] will be applied to design a new method for estimating the likelihood of cell survival using global network 'indicator states' linked to a particular cell fate, survival or death. The predictive models will be integrated with other components of the integrated ContraCancrum simulator.

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A7. Biomechanical models of tumours and adjacent normal tissue

A7.1. State of the art

Soft tissue simulations represent a challenge in biomechanical modelling. Soft tissues are highly nonlinear viscoelastic materials, and attempts to model their mechanical behaviour have led to different kinds of mathematical relationships depending upon the approach adopted. In addition, the mechanical properties of biological structures are highly complex and scarcely investigated. In ContraCancrum, finite-element-based modelling approaches for *brain* and *lung* biomechanics simulations will be developed and applied to study cancer biomechanics.

Traditionally, finite element modelling of the brain is focused on its dynamic behaviour during impact. The objective is to evaluate the brain injuries following high-energy trauma, as in a car accident [7-1]. These models focus on the viscoelastic properties of the brain tissue which are of utmost important for dynamic effects. With the introduction of neuronavigation, image registration systems and robotic neurosurgery, enthusiasm for brain modelling became more pronounced [7-2]. Recently, finite element models of the brain tissues have also been used to provide constraints to medical image registration [7-3]. In this work, a fully nonlinear (i.e. accounting for both geometric and material nonlinearities) patient-specific finite element brain model was applied to predict the deformation field within the brain during a craniotomy-induced brain shift. Deformation of brain surface measured on MRI images was used as a displacement boundary condition. However, in these cases, the biomechanical evaluation of stresses and strains in the tissues were not of primary importance.

Complete validation of any finite element model of the human brain is very difficult due to the lack of adequate experimental data. However, some experimental works describing the brain biomechanics in traction and compression are available [7-4, 7-5]. In addition, a visco-hyperelastic constitutive model of the brain has been proposed and successfully used to model brain shift [7-3]. This model is based on an Ogden strain energy function and includes relaxation of the shear modulus of the brain tissue.

Biomechanical finite element modelling of the lungs can be classified in two main categories; first models that focus on the detailed biomechanics of the alveolar tissue [7-6, 7-7] and second complex models of the thorax used for injury mechanisms due to automotive crashes [7-8] which has limited accuracy on the biomechanical behaviour of the lung tissues. At the full organ level, Ganesan [7-9] developed a finite element model of the dog lung, heart and abdomen, consisting of three solid linearly elastic bodies, to study the effects of gravity on the vertical stress distribution and lung volume in different body positions. The geometric representation of the different anatomic structure was extremely simplified in order to limit the model complexity.

Description of the mechanical properties of the lung is challenging. In most of the theoretical studies, the lung has been modelled as an isotropic and homogeneous material by using Hooke's constitutive law [7-10]. The hypothesis of homogeneous medium may be inappropriate for certain problems, because the foam-like structure and behaviour of the lung, if the air and soft tissue are considered as linear, can be assumed to behave like a linear elastic material. Some experimental measurements of the mechanical properties of the

tissue were conducted [7-11 to 7-13]. For example, Zhen measured the mechanical properties of human lung tissue in a state of biaxial tension. The experimental data were fitted with a pseudo-elastic constitutive equation. Other studies investigated the mechanical properties of the lungs based on its composition (collagen, elastin). The data, together with the known mechanical properties of collagen and elastin fibres, can be used to derive the incremental elastic moduli of the lung tissue.

During recent years, simplified tumour biomechanics have been used for atlas-based registration of a brain with tumours. Biomechanical models were used to determine the brain geometry prior to tumour development [7-14] and afterwards to evaluate the amount of deformation generated within different tissues by the tumour evolution [7-15, 7-16]. The focus of these models is the current state of the tumour and not the prediction of its development and the evolution of its shape during growth. Wasserman et al. [7-17] published one of the first works on biomechanical simulation including tumour growth. They proposed a general framework that can be employed to predict the direction and extent of spread of a primary brain tumour with respect to time and for a specific patient. Finite element simulations were used to calculate to stress within the tissues. A 2D validation of the model on one patient was presented.

More recently, Glatz et al. [7-18] proposed a patient-specific simulator of glioblastoma multiform (GBM) growth including the brain deformation induced by the tumour invasion. The simulation relies on a model discredited with the finite element method initialised from the patient MRIs. Additional structures (such as the white matter fibre directions, gray matter) have been included in the patient model using an atlas. Glatz followed a similar approach to Wassermann but separated the glioblastoma growth into a proliferation component and a diffusion component. These two components are treated separately in the model, although a clear delimitation between proliferations types is difficult. Validation of the model was conducted on MRI images of one patient.

Further information on the state of the art in biochemical modelling of tumour response to treatment can be found in references [7-19 to 7-27].

A7.2. Expected results

Biomechanical parameters affect the macroscopic geometry of tumours. While tumours growing in homogenous environments maintain a spherical shape, the *in vivo* situation is fundamentally different. A commonly accepted hypothesis is that *in vivo* growth occurs along preferential orientations based on the directions of least resistance in the tissue. The method to be developed in this work-package will not restrict the possible shape of the tumour to analytic geometries, but aims to predict the final shape and extent of the tumour in the brain with and without therapeutic interventions.

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A8. Image processing techniques in oncology

A8.1. State of the art

(a) The generic setting

Combining morphological imaging, e.g. Computer Tomography (CT) or Magnetic Resonance Imaging (MRI), with functional imaging, e.g. Positron Emission Tomography (PET), has significantly boosted our capability in cancer diagnosis and therapy [8-1]. Incorporating PET has proved to have a profound impact on patient management, often leading to significant changes in subsequent cancer therapy. Over recent years, impressive clinical evidence is accumulating to indicate that PET imaging might serve also as a surrogate marker to monitor early response to cancer therapy [8-2]. On these occasions, the medical community expresses its rapidly growing need for standardised, reproducible, and quantitatively correct imaging. Three examples may suffice to illustrate this trend:

(i) PET imaging with fluorodeoxyglycose (FDG) as tracer substance is used to monitor the metabolic activity in the human body. In combination with CT, it has significantly improved the specificity in early diagnosis of aggressively growing tumour cells. First clinical studies provide impressive evidence that FDG can also be used for assessing response to chemotherapy much earlier than with the conventional morphology-based RECIST (Response Evaluation Criteria in Solid Tumours) criteria. In addition, FDG has been successfully used for defining biological target volumes (BTV) for radiation therapy by precisely delineating actively growing parts of large tumours and increasing radiation dose over these areas.

(ii) Similarly, PET imaging with fluorothymidine (FLT) as tracer substance is used to more specifically monitor cell proliferation in the human body and has been used to monitor recurrent tumour cells in the human body.

(iii) Hypoxia has been monitored in the human body by PET imaging with fluoromisonidazole (FMISO) as a tracer substance. Hypoxic areas in tumours are known to be more radiation resistant than other cells and require dose boosting in radiation therapy.

Advanced computerised image processing and analysis are needed to make optimal use of the above, not only to manage the sheer flood of data emanating from the latest generation of fast high-resolution scanners, but also to put the analysis of these images on to objective and more reproducible grounds so that results become more closely comparable among different applications, specialists and institutions. Two closely related image processing disciplines are involved: segmentation and registration (Figure A8-1).

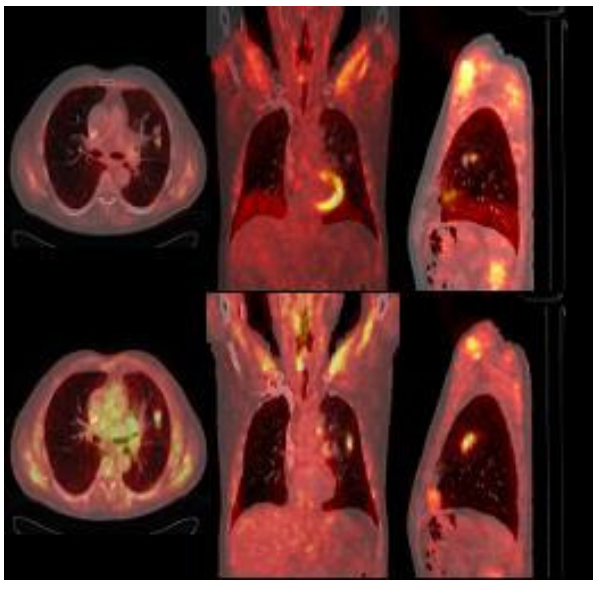

Figure A8-1. Colour-coded PET image overlaid on a CT image after rigid (*top row*) and elastic (*bottom row*) registration. The images were acquired independently of each other.

Note the different body and lung outlines in the top images due to the different breathing status of the patient. Elastic registration compensates for the breathing motion and aligns not only the lung volumes, but also a hot spot in the PET image with a lesion in the CT image indicative of a malignant, i.e. cancerous, tumour (from [8-3]).

Registration: In all of the above application examples, a single image does not tell the whole story; for that, several images have to be analysed in combination. These images can be acquired with the same or different imaging modalities, e.g. CT, PET, MRI etc., simultaneously or at different times, and can come from the same or different patients. Unless acquired simultaneously, anatomical structures are likely to have changed their positions between the acquisitions due to patient or organ motion, tumour growth or shrinkage, breathing motion, heart motion etc. Combining such images requires that they undergo geometric warping so that corresponding image structures correctly align. Aligning images for combined visual inspection (image fusion) is, however, only one of many applications of registration in medical imaging. More often and less visibly, registration techniques are deployed for motion compensation during acquisition and reconstruction of medical images as well as segmentation and classification of medical. Images can be registered by aligning anatomical landmarks, e.g. organ boundaries, that have been segmented from the images to be aligned (feature-based registration) or directly aligning the original intensity values (volume-based registration).

Segmentation: Segmentation identifies areas in a digitised image containing image elements that are related to each other with respect to some underlying property, e.g. belonging to the same organ, being malignant tumour cells, showing similar tracer uptake, etc. Besides mere identification, extracting quantitative information further characterising the delineated segments is required, e.g. to determine the diameter of a tumour, the tracer accumulation within the tumour volume, the oxygen level, etc. Different classes of algorithms representing different methodological approaches have been developed over the last decades. The approaches range from *region growing* (e.g. watershed or front propagation algorithms), over *statistical classification* (e.g. Markov random fields Bayesian classifiers, support vector machines) to *model-based approaches*, which adapt general anatomical or physiological models to the data sets acquired from individual patients by using, for example, active contour techniques.

Each of the above areas is still an area of active research in academia and industry. Many different approaches have been proposed and investigated – the three-volume Handbook of Medical Imaging [8-4] provides a comprehensive overview of the most established ones. All major vendors in the field of medical imaging as well as many institutions in the public domain offer packages of computer algorithms that seek to address problems in this area. These are very different in their capabilities, maturity level and application range. A common feature, however, is that none of them provides a complete solution successfully handling all of the aspects involved in multi-modality diagnosis and therapy. This requires a thorough understanding of the clinical target application combined with an equally thorough understanding of the capabilities of the basic algorithmic blocks from which dedicated solutions can be built and validated.

(b) The ContraCancrum specific setting (the brain case)

Image registration of pathological brain images is a demanding task due to the complexity of the underneath pathology. This complexity increases when patient follow-up is required. Therefore, correct description of tumours has been shown to improve the success of the registration process. This description must take into account the deformation produced by the tumour physiology in and around the tumoural area. Whereas readily available registration algorithms produce good results for healthy images, they fail at describing the correct deformation produced by tumours owing to their inability to describe tissue death, tumour infiltration, tumour mass-effect, etc.

Several approaches have been presented to counter these pitfalls. In [8-5], a biomechanical model of tumour-induced deformation was proposed to register 2D images of non-infiltrative tumour patients to an atlas. Later, in [8-6], the introduction of tumour "seeds" in the atlas

image combined with image features to drive the registration was proposed, and this was later extended in [8-7] by adding a geometrically symmetric model of the tumour. A more sophisticated model was recently presented in [8-8]; here, a statistical model of tissue deformation due to tumour physiology is incorporated into a 3D non-rigid brain image registration framework. The statistical model is used to tune the tumour model parameters and guide the registration of a healthy brain image (e.g. atlas) and a brain tumour image. Interesting work on the alignment of volume MR images and high resolution [18F] Fluorodeoxyglucose PET images for the evaluation of patients with brain tumours is presented in [8-9].

A8.2. Expected results

The consortium will provide an ideal combination of expertise in the acquisition and analysis of medical images and the simulation of cancerous processes. A strong and focused interaction of experts in both fields is expected to take image processing a step further in the clinical setting by enhancing it with computational cancer biology and oncology.

Image segmentation will be extended by using already available brain atlases, which combined with the multilevel biological and biomechanical tumour model, will bring a segmented atlas adapted to the patient anatomy and the specific tumoural pathology. Although the methodology is sought to be as fully automatic as possible, focus will be given to incorporate manual editing of the segmentation so to add flexibility and to ease the correction of the segmentation obtained.

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Appendix B. Embryological Development of the Brain

The normal development of the brain starts with the thickening of the dorsal ectodermal surface of the early embryo which will elongate to form the neural plate at day 16. Subsequent changes convert the plate into a neural tube. Cells lining the cavity of the neural tube are neuroepithelial cells which ultimately give rise to all the cells of the CNS. The anterior end of the neural plate enlarges and will form the brain while the caudal portion of the neural plate will form the spinal cord. The cavity of the developing neural tube is destined to become the ventricular system. Ectoderm at the margins of the neural folds represents cells destined to become neural crest cells that form clusters. These dorsally located cells migrate peripherally to become sensory ganglia of the cranial and spinal nerves, autonomic and enteric ganglia, Schwann cells, melanocytes, adrenal chromaffin cells, and the pia/arachnoid membranes, whereas the dura develops from the mesoderm.

By about 25 days the cephalic and caudal closure of the neural tube becomes complete. The cephalic closure of the neural tube is marked by the lamina terminalis of the adult. After closure of the neural tube, the expanded rostral portion of the neural tube will differentiate and subdivide into three vesicles representing the forebrain (prosencephalon), midbrain (mesencephalon) and hindbrain (rhombencephalon). These bends are due to tremendous cell proliferation, differential growth and because the brain develops in the confined space of the cranial vault. By about the end of the 3rd week, this 3-part brain begins to assume a "C"-shape by the formation of cephalic flexure at the level of the mesencephalon. At the end of the 4th week, a cervical flexure develops between the hindbrain and spinal cord and the 3-part brain begins to develop 5 vesicles (Fig. B1). The forebrain (prosencephalon) gives rise to the paired lateral telencephalic vesicles, which bud off from the prosencephalon and will become the cerebral hemispheres, and the diencephalon, from which the optic vesicles extend.

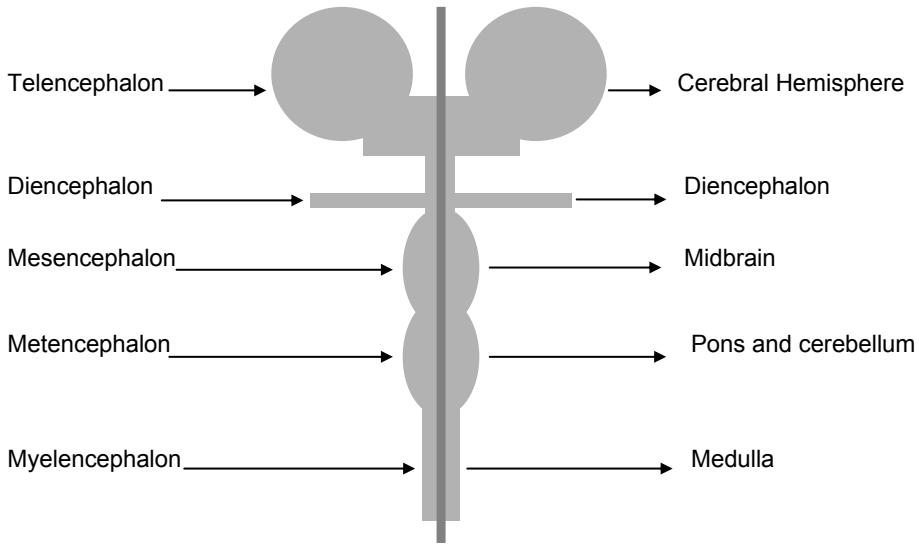

Figure B1. Five vesicle stage of brain development

At the cephalic flexure, the mesencephalon remains tubular and undivided. The hindbrain (rhombencephalon) subdivides into a metencephalon and a more caudal myelencephalon. In the 5-vesicle stage (6th week), the pontine flexure develops in the rhombencephalon which divides it into a metencephalon and myelencephalon. The metencephalon is more cranial and forms the pons and cerebellum; the myelencephalon becomes the medulla. The presumptive site of the cerebellum is seen as the rhombic lips at the cranial edge of the thin roof of the 4th ventricle. A depression develops in the prosencephalon which defines the telencephalon

from the diencephalon. Subsequent growth of the telencephalon will cause it to expand dorsally, caudally, laterally, and inferiorly.

As these enlargements appear, the brain begins to curve in certain areas (Fig. B2). There is a cephalic flexure that appears in the region of the midbrain or mesencephalon. The pontine flexure is located at the junction between the metencephalon and the mylencephalon. It will eventually give rise to the rhombic lip of the metencephalic alar plate and then become the cerebellum. There is also a cervical flexure that appears at the medulla-spinal cord junction.

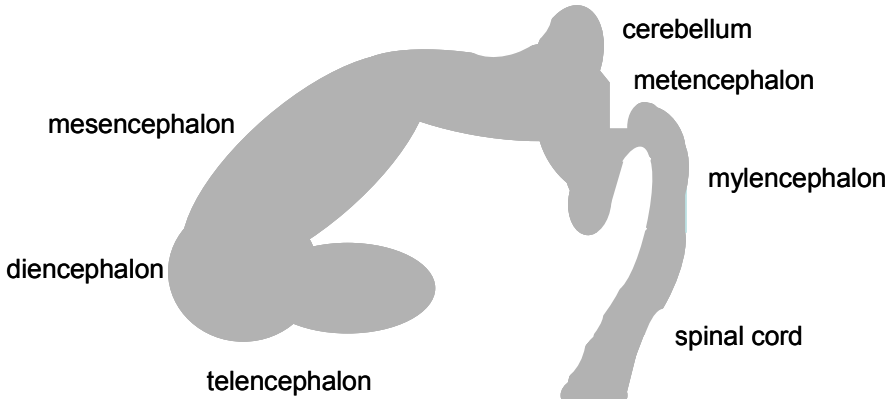

Figure B2. Formation of flexures

The internal central canal of the neural tube in the region of the brain enlarges and, except for the **midbrain**, develops into ventricles. The ventricles will contain the cerebrospinal fluid. The ventral horn cells of the spinal cord constitute a cell column that runs the length of the cord and contains somatic efferent neurons that are involved in motor innervation to skeletal muscles. Sensory neurons of the spinal cord form cell columns in the dorsal horn of the spinal cord. Finally, autonomic motor neurons form a cell column that is located in the lateral gray horn and is located from the T1 (first thoracic) to the L2 (second lumbar) vertebra.

The **mylencephalon** develops into the medulla. The caudal end of the mylencephalon remains closed with a central canal continuous with the central canal of the spinal cord. As the more cephalic end of the medulla is reached, the roof of the ventricle opens and is drawn out as the roof plate. Together with the pia, the roof plate gives rise to the tela choroidea. Heavily vascularised parts of the tela choroidea project into the 4th ventricle as the choroid plexus.

The **low medulla** contains a central canal and the cell columns that develop are for the hypoglossal nerve (CN12), the vagus nerve (CN10) and the sensory nuclei for the sensory nuclei for cranial nerves 10 and 5 (the trigeminal nerve). New pathways that ascend and descend through the spinal cord are located posterior and lateral in the low medulla.

The **high medulla** sees some changes. As mentioned above the fourth ventricle opens. Cell columns for the vestibular nuclei and the cochlear nuclei appear. The sensory nuclei for cranial nerve nuclei 5, 9 (glossopharyngeal nerve) and 10 also are found. Motor nuclei for the glossopharyngeal and vagus nerves as well as the 12th cranial nerve are also present. The long ascending and descending tracts have assumed a more anterior and lateral position.

The **metencephalon** develops into the cerebellum and the pons. The cephalic part of the fourth ventricle continues into the pons. The roof plate becomes the superior medullary velum a region of the pons that will be covered by the cerebellum. During the formation of the cerebellum, the mantle layer of the rhombic lip will develop into the deep nuclei of the cerebellum. It will also give rise to the cells that will form the cerebellar cortex.

The **cell columns** of the pons include those for the sensory nuclei for the vestibulocochlear nerve, the trigemminal nerve, the facial nerve and the motor nuclei for the facial nerve, the trigemminal nerve and the abducens nerve. Descending pathways are located in the base of the pons along with pontine nuclei. Other ascending pathways are located in the anterolateral pons, and the middle and superior cerebellar peduncles.

The **mesencephalon** changes very little during development. The central canal is small and is now called the cerebral aqueduct. The roof plate and the alar plate become the tectum and contain nuclei associated with vision and with hearing. The floor and the basal plates become the cerebral peduncles.

The cell columns associated with the **midbrain** include those associated with the eye muscles as well as with autonomic function. The newer ascending and descending pathways are found along the anterolateral tegmentum and crus cerebri portions of the midbrain. This part of the brain develops into the thalamus, the epithalamus, the subthalamus and the hypothalamus. The central canal forms the third ventricle. The roof plate forms the tela choroidea and choroid plexus and is continuous with the lateral ventricle via the interventricular foramina of Monroe.

The **diencephalon** does not have a basal plate. The alar plate divides into a dorsal region and a ventral region and the dividing line is the hypothalamic sulcus. The thalamus is located in the dorsal region and constitutes a major set of relay nuclei for pathways involved in sensory function. The ventral region is comprised of hypothalamic nuclei. These nuclei are involved in the regulation of visceral function.

During development, the **telencephalon** expands significantly. The telencephalon hemispheres flex into a "**C-shaped**" structure. The mantle layer gives rise to the cells of the basal ganglia, a region of the brain that regulates motor function. The mantle layer also gives rise to cells that will migrate into the marginal layer, and these cells become the cortical gray cells. During development, the medial walls of the telencephalon fuse with the lateral walls of the diencephalon. The internal capsule forms at the line of fusion. The growth of the telencephalic hemispheres causes them to grow over the basal ganglia and the cortex over it called the insular cortex. The central canal of the telencephalon forms the lateral ventricle. The choroid plexus develops along the medial wall of the lateral ventricle. A corpus callosum forms as a group of fibres that connect one hemisphere to the other.

During histogenesis, ectodermal cells of the early tube develop 3 concentric zones:

- germinal (or matrix),
- mantle, and
- marginal

Cells of the original single-layered tube divide to form a neuroepithelium the cells of which extend from the neural canal to the external surface of the tube. Cells near the central canal are called the germinal layer. They divide rapidly, thickening the walls of the tube and eventually producing neuroblasts and glioblasts. Newly formed undifferentiated cells of the neuroepithelium migrate outward forming a 3-layered tube consisting of

- an internal, columnar ependymal layer, which becomes the ependymal lining and epithelium of choroid plexus,
- a middle, densely packed layer of mantle cells, which becomes the gray matter of the CNS,
- an external marginal layer composed mainly of the processes of cells of the mantle layers, which becomes the white matter of the CNS.

Appendix C. Molecular Biology of Gliomas

Table C1. Proto-oncogenes involved in gliomas

Gene	Location	Typical alteration	Function of the protein	Common in
EGFR	7p11	Amplification and overexpression, genomic rearrangement	Tyrosin kinase growth factor receptor	Glioblastoma (GBM)
PDGFR	4q12	Amplification and overexpression	Tyrosin kinase growth factor receptor	GBM
MET	7q31	Amplification and overexpression	Tyrosin kinase growth factor receptor	GBM
CDK4	12q13	Amplification and overexpression	Cyclin-dependent kinase, promotes G1/S phase progression	GBM
CCND1	11q13	Amplification and overexpression	Cyclin D1, promotes G1/S phase progression	GBM
CCND3	6p21	Amplification and overexpression	Cyclin D3, promotes G1/S phase progression	GBM
MDM2	12q15	Amplification and overexpression	Inhibitor of p53 function	GBM
MDM4	1q32	Amplification and overexpression	Inhibitor of p53 function	GBM
MYCC	8q24	Amplification and overexpression	Transcription factor	GBM

Table C2. Common tumour suppressor genes involved in gliomas

Gene	Location	Typical alteration	Protein Function	Commonly Seen In
TP53	17p13	Mutation	Involved in the regulation of apoptosis, cell cycle progression, DNA repair	Anaplastic astrocytomas, glioblastoma (secondary > primary)
PTEN	10q23	Mutation	Protein phosphatase and lipid phosphatase, negative regulator of phosphatidylinositol 3-kinase	Glioblastoma
CDKN2A	9p21	Homozygous deletion	Inhibitor of cyclin dependent kinase 4 and 6	Glioblastoma
RB1	13q14	Mutation hypermethylation	Nuclear phosphoprotein involved in cell cycle regulation	Glioblastoma, Anaplastic astrocytomas
p14 ^{ARF}	9q21	Homozygous deletion hypermethylation	Inhibitor of Mdm2	Anaplastic astrocytomas

Table C3. Common genetic aberrations in familial astrocytic tumours

Syndrome	Gene	Location	Function	Tumour type
NF-1	NF1	17q11.2	GTPase activating protein homology	Astrocytic tumours (brain stem optic n.) ependymomas
NF-2	NF2	22q12.2	Ezrin/Moesin/Radixin-like	Vestibular Schwannomas, gliomas
Li Fraumeni	TP53	17p13.1	Transcription factor Apoptosis inducer	Astrocytic tumours
Tuberous sclerosis	TSC1/2	9q34/16p13.3	GTPase activating protein homology	Subependymal Giant cell astrocytoma
Turcot's syndrome	MLH1/PS2	3p21.3/7p22	MIN+	Glioblastoma
Cowden disease	PTEN	10q22-q23	Dual-specificity phosphatase and Tensin homology	Astrocytic tumours

Appendix D. Clinical Trials – Regulation and Practice

Clinical research is mainly based on clinical trials. These enable clinicians to find new and better ways to understand, detect, control and treat illness. Clinical trials will give answers to health related questions [D4].

The most commonly performed clinical trials evaluate new drugs, medical devices, biologics or other interventions on patients in strictly scientifically controlled settings, and are required for regulatory authority approval of new therapies. Trials may be designed to assess the safety and efficacy of an experimental therapy, to assess whether the new intervention is better than standard therapy, or to compare the efficacy of two standard or marketed interventions. The trial objectives and design are usually documented in a Clinical trial protocol. While in a clinical trial, participants following a protocol are seen regularly by the research staff to monitor their health and to determine the safety and effectiveness of their treatment.

Clinical trials can be categorised according to the type of sponsor for the trial. Investigator-initiated trials have to be distinguished from trials that are sponsored by pharmaceutical companies. In particular, investigator-initiated trails lack commercial funding and thus have low logistic and financial support. Most of these trials are aimed at optimising treatments in a clinical setting.

While the term clinical trial is most commonly associated with large randomized studies, many clinical trials are relatively small. Clinical trials can be differentiated into the following categories:

- treatment trials test experimental treatments, new combinations of drugs or new approaches to surgery or radiation therapy;
- prevention trials search for better ways to prevent disease in people who have never had the disease or to prevent a disease from returning; these approaches may include medicines, vitamins, vaccines, minerals, or lifestyle changes;
- diagnostic trials are conducted to find better tests or procedures for diagnosing a particular disease or condition.
- screening trials test the best way to detect certain diseases or health conditions.;
- Quality of Life trials (or Supportive Care trials) explore ways to improve comfort and the quality of life for individuals with a chronic illness.

The value of clinical trials as the optimum methodology for testing and evaluating new treatments and medicines is well recognised within the research community. Clinical trials conducted on human participants are designed and conducted according to sound scientific and ethical standards within the framework of good clinical practice. Compliance with these standards provides the public with assurance that the rights, safety and well being of trial participants are protected and that the clinical trial data are credible. Guidelines for clinical trials should be read in the context of the Declaration of Helsinki, October 2000 and the ICH Harmonised Tripartite Guidelines for Good Clinical Practice, May 1997 [D1].

Several more or less mandatory guidelines exist for clinical trials. In the following the most relevant regulations and guidance/guidelines regarding CDM process will be mentioned.

ICH – Good Clinical Practice [D1]

ICH has published a variety of guidelines that are divided into four major categories; ICH Topic Codes are assigned according to these categories:

- Q: "Quality": related to chemical and pharmaceutical Quality Assurance.
- S: "Safety" related to *in vitro* and *in vivo* pre-clinical studies.

- E: "Efficacy" Topics, related to clinical studies in human subject, for example: E6 Good Clinical Practices.
- M: "Multidisciplinary" related to cross-cutting topics that do not fit uniquely into one of the above categories.

Clinical trials compliant to the ICH guidelines are normally accepted worldwide, because ICH is a project of the most important regulatory agencies and pharmaceutical companies. Most of the guidelines summarised under the topic "Efficacy" and two of the guidelines under the Topic "Multidisciplinary" are related to clinical data management practices. Four of them will now be described in more detail as these most affect clinical data management.

- ICH Harmonized Tripartite Guideline General Considerations for Clinical Trials (E8) (ICH 1997).

  This document is intended to give an overview of the ICH clinical and safety guidelines for readers who are unfamiliar with the ICH requirements.
- ICH Harmonized Tripartite Guideline for Good Clinical Practice (E6 (R1)) (ICH 1996)

  This sets forth an international ethical and scientific quality standard for the conduct of clinical trials that involve the participation of human subjects. The GCP guideline covers all aspects of preparation, design, monitoring, recording, reporting and archiving of clinical trials.
- ICH Harmonized Tripartite Guideline Clinical Safety Data Management: Definitions and Standards for Expedited Reporting (E2A)

  This guideline falls under the broad topic of Clinical Safety Data Management. These guidelines provide standard definitions, an international medical terminology (via the Medical Dictionary for Drug Regulatory Affairs – MedDRA) and standard data elements for reporting medical information as well as timeframes for reporting safety information to regulatory authorities.
- ICH Harmonized Tripartite Guideline Structure and Content of Clinical Study Reports (E3) (ICH 1995)

  This guideline is intended to facilitate the compilation of a single worldwide core clinical study report acceptable to all regulatory authorities.

EU Directives

A European Union (EU) Directive was published in May 2001 – The EU Directive on Clinical Trials (2001/20/EC); this can be downloaded directly[3]. In addition a second directive was published in 2005 (2005/28/EC), which can also be directly downloaded[4].

The EU Clinical Trials Directive regulates the conduct of clinical trials involving medicines for human use. The aims of the directive are:

- to protect the rights, safety and well being of trial participants
- to simplify and harmonise the administrative provisions governing clinical trials
- to establish a transparent procedure that will harmonise trial conduct in the EU and ensure the credibility of results.

The directive means that all interventional clinical trials, whether commercially funded or non-commercial, must meet these requirements. The EU Directive will have a number of implications for all parties involved in clinical trials. It enforces a number of new legal obligations for Chief/Principal Investigators and trial sponsors. In most commercially funded

[3] http://www.wctn.org.uk/downloads/EU_Directive/Directive.pdf

[4] http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2005:091:0013:0019:EN:PDF

studies, the commercial company takes the role of 'sponsor' and is therefore responsible for the conduct of the study. The Directive has particular implications for studies funded by charities and studies funded by other non-commercial organisations.

Compliance with the above-mentioned standards provides public assurance that the rights, safety and well-being of trial subjects are protected, in a way consistent with the principles that have their origin in the Declaration of Helsinki, and that the clinical data are credible. The following principles have to be strictly followed [D4]:

- Clinical trials should be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and the applicable regulatory requirements
- Before a trial is initiated, foreseeable risks and inconveniences should be weighed against the anticipated benefit for the individual trial subject and society. A trial should be initiated and conducted only if the anticipated benefits justify the risks
- The rights, safety, and well-being of the trial subjects are the most important considerations and should prevail over the interests of science and society
- The available non-clinical and clinical information on an investigational product should be adequate to support the proposed clinical trial
- Clinical trials should be scientifically sound, and described in a clear, detailed protocol
- A trial should be conducted in compliance with the protocol that has received prior ethics committee approval
- The medical care given to, and medical decisions made on behalf of subjects should be the responsibility of a qualified physician
- Each individual involved in conducting a trial should be qualified by education, training and expertise to perform his or her respective tasks
- Freely given informed consent should be obtained from every trial participant prior to clinical trial participation
- All clinical trial information should be recorded, handled, and stored in a way that allows its accurate reporting, interpretation and verification
- The confidentiality of records that could identify subjects should be protected, respecting the privacy and confidentiality rules in accordance with the applicable regulatory requirements.

There is a variation between different countries in Europe in interpretation of the directive and the common problems encountered by established academic (non-commercial) trial groups in maintaining and opening new multinational clinical trials for cancer.

The practical application of these principles requires that clinical trials have distinct components built into them. These include [D4]:

1. *Relevant and appropriate study rationale*

   A study rationale and motivation which does not ask relevant and important questions is unethical. Whilst maintaining the highest standards of clinical research it is important that clinical trials are based on priority research questions. Relevant and important questions should be problems that significantly affect local and regional population. Study rationale should demonstrate that the study question has not been substantially answered and that adequate systematic review of the subject under discussion was done.

2. *Optimal study design*

   Appropriate designs are critical in contributing to answering scientific questions. The design must therefore demonstrate a high probability for providing answers to

specific research questions. Adequate supporting information and explanation on the study sample size and study population must also be provided.

3. *Investigator competence*

   The investigator's competence is assessed by two major parameters: Technical and humanistic. Technical competence which includes research competence is assessed by education, knowledge, certification and experience. Humanistic parameters require compassion and empathy. This is provided by a proper clinical and research environment.

4. *Balance of risks and benefits for participants*

   A risk benefit analysis of the study should precede the conduct of the research itself. Risk-benefit analysis should take full cognisance of benefits and harms beyond the life of the study itself, particularly in the case of chronic life threatening conditions. Alternative ways of providing benefits to the patients might be available without research; thus the distinction between the probability of harm and the possible benefits of the effects must be made.

5. *Transparency*

   A clinical trial in the European Community can only obtain approval, if the trial information is registered in the EudraCT database, which is centralized in UK for Europe. The database will serve to promote transparency to prevent unnecessary trials.

6. *Patient privacy*

   The patient's privacy, data protection and security are essential tasks in clinical trials.

7. *Ethics*

   In respect to ethics clinical trials have to pass ethical committees and regulatory or legal authorities. In ongoing trials data and safety monitoring committees are included.

   - Ethical Committees. These are usually made up of lawyers, medical practitioners, bio-ethicists and community representatives
   - Data and Safety Monitoring Committees (DSMC). These committees oversee ongoing clinical trials with respect to treatment, efficacy and safety. In the advent of clear evidence of efficacy or harm, prior to the end of the trial, premature termination can be recommended on ethical grounds
   - The Regulatory Authority which is responsible for reviewing the study design

8. *Impartial oversight of consent procedures*

   Informed consent is a necessary but not sufficient requirement for ethical conduct. Obtaining informed consent implies the provision of information to potential participants regarding the nature of the research procedure, scientific purpose and alternatives to study participation. Participants' comprehension is addressed by laying out this information in a clear and simple style, including the use of the participant's language. The conditions under which the consent is granted must be free of coercion, undue influence or incentives. Treatment for a given condition, which might be an attribute of the clinical trial design, should not be denied by the refusal to participate. Withdrawal from the clinical trial at any time will not result in undue clinical penalties to the participant.

9. *Safety monitoring*

   Safety monitoring of participants during and for defined periods after a clinical trial is an ethical requirement. This involves the prompt reporting of serious adverse events and the appropriate management of such an event. (SAE: Severe adverse events, SUSAR: Suspected unexpected severe adverse reaction)

As a result of the Clinical Trials Directive 2001/20/EC the conduct of clinical trials throughout Europe has changed [D2, D5]. The directive, aimed largely at holding pharmaceutical companies to higher standards, has tied up academic clinical research, particularly large trials, with redundant paperwork, liability tangles and unending bureaucracy [D2].

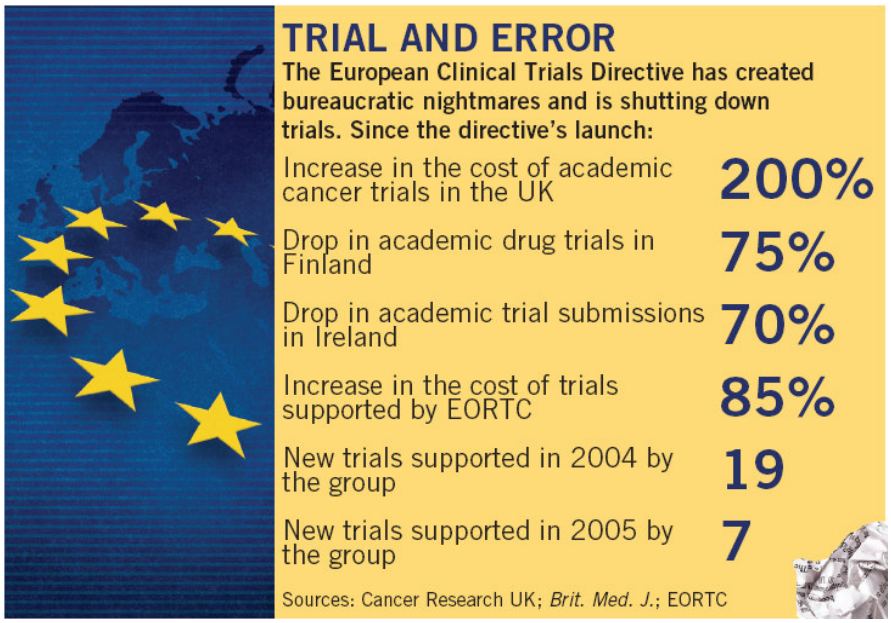

Figure D1. The impact of the European Clinical Trials Directive 2001/20/EC, (from [D2])

Brandon Keim [D2] writes in Nature Medicine: "The cost of academic cancer trials has doubled since 2004**,** according to Cancer Research UK, the country's largest sponsor of academic cancer research. The European Organization for the Research and Treatment of Cancer estimates that expenses have risen by 85% and says the number of trials it supports has dropped by 63%. The Save European Research campaign, which represents more than 3,000 scientists, says academic drug trials have dropped by 70% in Ireland and 25% in Sweden. The number of Finnish academic drug trials shrunk by 75%". [D2]. One of the biggest bottlenecks is the directive's requirement that each trial has to have a single sponsor who is fully liable for all legal and financial issues. For trials running in different European Countries the problem of a single sponsor is not solved yet. Key issues for Cancer Trials are summarized by Kathy Pritchard-Jones in the European Journal of Cancer [D3]. Though this article deals with clinical trials for children, most of these points are relevant for clinical trials in adults.

To enrol more patients in clinical trials, the following points will have to be reconsidered by the European Commission [D3]:

- The bureaucratic workload of trial activation has to be reduced
- The content and implementation of the EU Clinical Trial Directive has to be reappraised, especially for European study groups who run multinational clinical trials.
- For multinational trials a single application system for clinical trial authorisation is needed, whereby the clinical trial application is managed by a single competent authority rather than up to 27 national competent authorities.
- There is an urgent need to reappraise how the responsibilities of the sponsor are implemented. Consideration should be given to provide the necessary support at a European and/or national level to allow academic institutions or governmental research bodies to feel comfortable with taking on the role of European sponsor for investigator-led trials.

- Any further adaption of regulatory requirements should be based on the risk of the trial rather than on what category of researcher a trial was initiated. Investigator-initiated trial must be supported (including finances) and made feasible within the resources of academic networks.

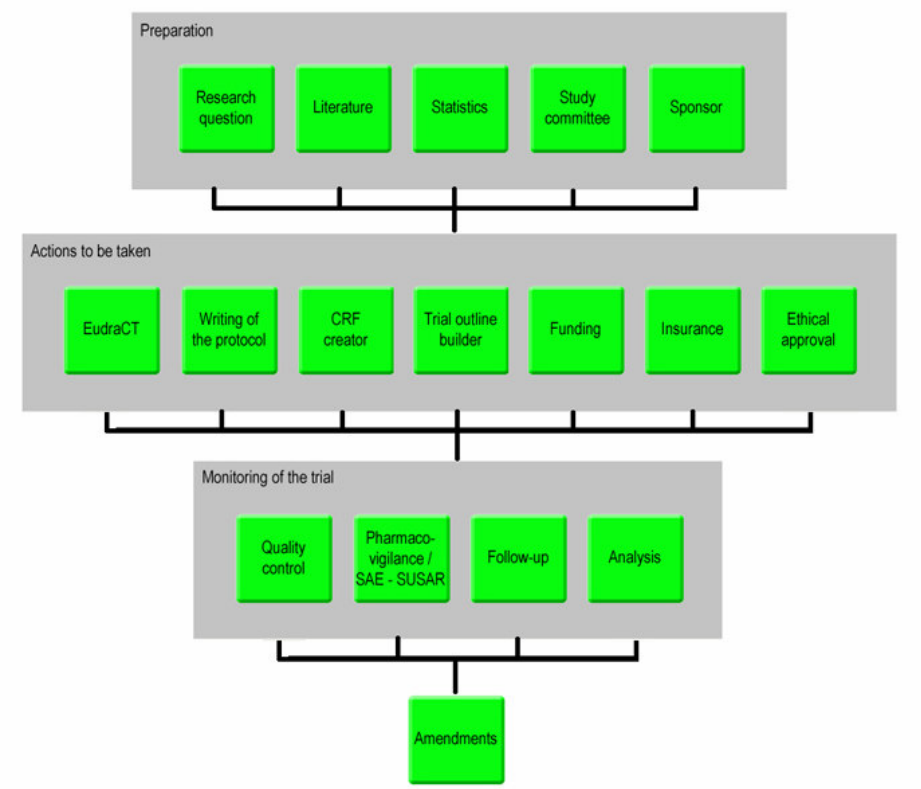

Figure D2. Roadmap of clinical trials

A roadmap of the conduct of a clinical trial is given in Figure D2. One can identify different phases:

1. The preparation of the trial
2. Actions to be taken before running the trial
3. Running and monitoring the trial including amendments

Regarding both scenarios (glioma, lung cancer) actions are taken to get clinical trials running. The following data will be provided by the clinical partners of USAAR:

Gliomas:

a. Clinical data of patients
   - Age, gender, symptoms, neurological findings, Karnofsky Index, date of diagnosis
   - surgery (yes or no), if yes : date of surgery, type of surgery (biopsy, subtotal resection, gross total resection), Karnofsky after surgery
   - Irradiation (yes or no), if yes: start and end of Radiotherapy, treatment schema, single dose, cumulative dose, field,
   - Chemotherapy (yes or no), if yes: start and end of chemotherapy, chemotherapeutic protocol, names and dosages (single and cumulative) of drugs
   - Other drugs, e.g. steroids (name of drug, dosage, duration)
   - SAEs, SUSARs
   - Remission status after surgery, after irradiation at the end of treatment
   - Karnofsky Index during treatment and at end of treatment

- Date of relapse, kind of relapse
- Date of death, reason of death

b. Imaging studies
- DICOM data at time of diagnosis, after surgery, end of treatment
  – it is necessary to define imaging parameters
  – a tool for tumour rendering has to be provided

c. Histopathological diagnosis

d. Pattern of autoantigens from sera

Lung Cancer:

a. Clinical data of patients
- Age, gender, symptoms, neurological findings, Karnofsky Index, date of diagnosis
- surgery (yes or no), if yes : date of surgery, type of surgery (biopsy, subtotal resection, gross total resection), Karnofsky after surgery
- Irradiation (yes or no), if yes: start and end of Radiotherapy, treatment schema, single dose, cumulative dose, field,
- Chemotherapy (yes or no), if yes: start and end of chemotherapy, chemotherapeutic protocol, names and dosages (single and cumulative) of drugs
- Other treatment, e.g. targeted therapy (name of drug, dosage, duration)
- SAEs, SUSARs
- Remission status after surgery, after irradiation at the end of treatment
- Karnofsky Index during treatment and at end of treatment
- Date of relapse, kind of relapse
- Date of death, reason of death

b. Imaging studies
- DICOM data at time of diagnosis, after surgery, end of treatment
  – it is necessary to define imaging parameters
  – a tool for tumour rendering has to be provided

c. Histopathological diagnosis including macropathological imaging

d. Results of mutation analysis and sequencing of EGFR

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