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ABSTRACT:

This deliverable describes the scenarios and data of the different cancer domains that will serve as input to drive the developments of tools and services in CHIC. The addressed clinical trials for nephroblastoma, glioblastoma multiforme and non-small cell lung cancer are explained by the clinician from the viewpoint as data provider for optimal understanding of the scenarios and data by the developers and to guarantee the clinical relevance of the end-product. For each of the three cancer domains the orientation of the clinical problem, the clinical scenario, the generated data, data storage and ethical considerations are described.

KEYWORD LIST:

Clinical scenario, generated data, data storage, ethical considerations, nephroblastoma, glioblastoma multiforme, non-small cell lung cancer

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[1] **R**=Report, **P**=Prototype, **D**=Demonstrator, **O**=Other

[2] **PU**=Public, **PP**=Restricted to other programme participants (including the Commission Services), **RE**=Restricted to a group specified by the consortium (including the Commission Services), **CO**=Confidential, only for members of the consortium (including the Commission Services)

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1 Executive Summary

In order to create clinical relevant tools, services and secure infrastructure, the CHIC project is clinically driven and clinically oriented by addressing and adopting three clinical trials. The multiscale data generated by these clinical studies will be exploited to drive the development of integrative multiscale hypermodels and hypermodel oncosimulators and to clinically adapt and validate them.

This deliverable is a report on scenarios and data from defined patients from the nephroblastoma SIOP-2001 trial, glioblastoma patients from the HGG-2010 trial and patients with non-small cell lung cancer, used for building meta- and hyper-multiscale models and repositories reused for the integrated hypermodel based oncosimulator. The clinical scenario, generated data, data storage and ethical considerations of the three trials are described here for optimal understanding by the users of the scenarios and data.

It is noted that the nephroblastoma and the lung cancer hypermodel based oncosimulators will primarily be based on mechanistic mathematical models whereas in the case of glioblastoma the hypermodel based oncosimulator is primarily based on machine learning techniques.

2 Introduction

2.1 Purpose of this document

The purpose of this document is to define the clinical scenarios - and the therefrom generated data - that will serve as the source of the input for the multiscale hypermmodels and the integrated hypermodel based Oncosimulator, from the point of view of clinicians as data provider. Hence, this deliverable describes the sources that drive the main objective of Work Package 3, which is the *validation of the CHIC environment by focussing on three different cancer types, nephroblastoma, glioblastoma multiforme and non-small cell lung cancer. Therefore clinical relevant cases are defined and their data need to be stored within the infrastructure of CHIC in a secure and anonymized way according to the legal and ethical framework of CHIC. The data from these concrete clinical scenarios will undergo processing within the environment, and validation of the environment will be based on the clinical and oncologic data produced by the same scenarios.*

To succeed in this objective a good understanding of the clinical scenarios and data is necessary as a basis for interaction between data providers (clinicians/researchers) and the basic scientists, IT people, modellers, legal people. An iterative process between clinicians as the drivers (and end-users) and the developers should take place to guarantee clinical relevance (as described in D2.5). In this document the clinicians provide a description of the aspects of which they are responsible for as data provider, namely the clinical scenarios and the scenario-based data as input for the developers.

From the basic science modelling perspective it is noted that the nephroblastoma and the lung cancer hypermodel based oncosimulators are primarily based on mechanistic mathematical models whereas in the case of glioblastoma the hypermodel based oncosimulator is primarily based on machine learning techniques. The selection of the particular modelling approaches has been dictated by the nature of the biomedical data in conjunction with the potential and the limitations of the mathematical and computational approaches available.

This deliverable is also the report of Milestone 5: *Scenarios and data from nephroblastoma, glioblastoma multiforme and non-small cell lung cancer are available*. This milestone has specific objectives:

- Definition of scenarios and evaluation criteria supported by CHIC (in collaboration with WP11: Milestone 27, 28, 29)
- Availability of data from nephroblastoma, glioblastoma and lung cancer patients
- Use of ObTiMA in a prospective clinical trial

In the following chapters each of the three cancer types will report on these items.

3 Nephroblastoma scenario and data

3.1 Background

In Europe patients with nephroblastoma are treated according to trials and studies of the International Society of Paediatric Oncology (SIOP). The main mission of the SIOP Renal Tumour Study Group (RTSG) is to increase survival and to reduce acute treatment toxicity and late effects in all children, adolescents and young adults diagnosed with any renal tumour. In this context SIOP-RTSG is aiming to offer all these patients the same standardized high quality diagnostics and treatment, independent of the tumour type, the socio-economic status or the geographic region the patient is living. To achieve these goals a new Umbrella protocol is under development. In this respect kidney cancer in childhood will serve also as a paradigm for other childhood cancers.

Renal tumours include nephroblastoma or Wilms tumours (WT) in around 90% of the cases. The other tumours comprise rare entities such as Clear Cell Sarcoma of the Kidney (CCSK), Renal Cell Carcinoma (RCC), Malignant Rhabdoid Tumors of the Kidney (MRTK), Congenital Mesoblastic Nephroma (CMN), and few others, even rarer tumours (Figure 3.1).

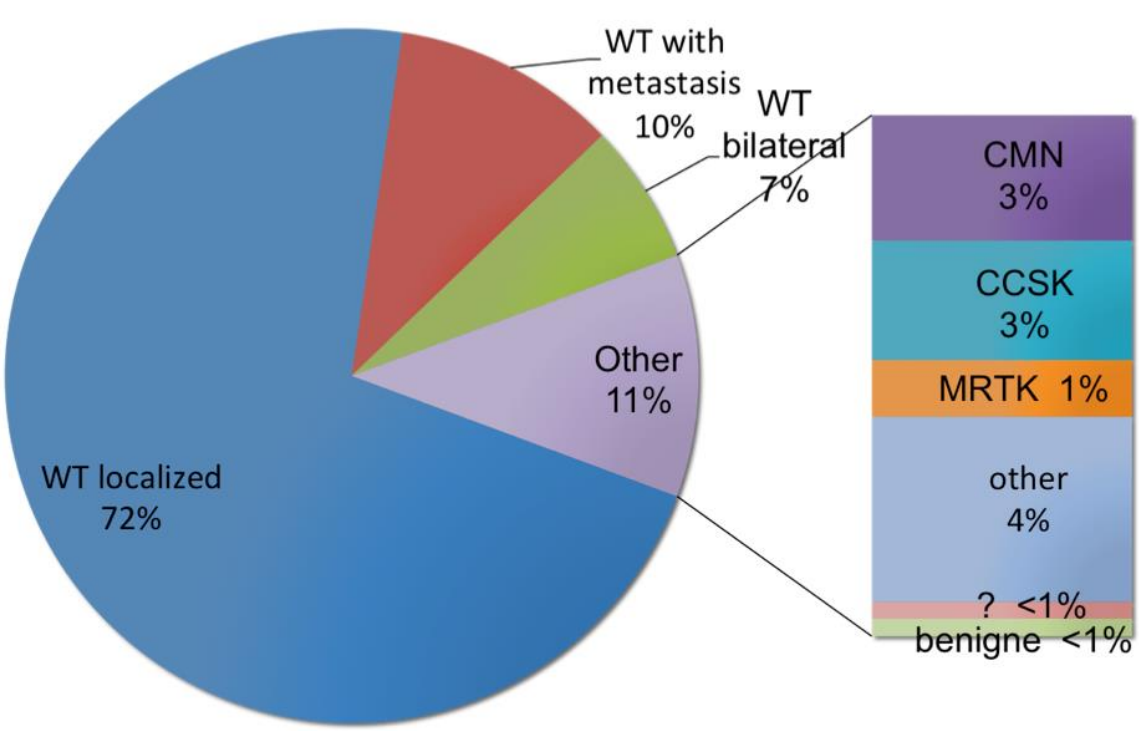

Figure 3.1: Distribution of renal tumours in childhood. (WT: Wilms tumour, CMN: congenital mesoblastic nephroma, CCSK: Clear cell sarcoma of the kidney, MRTK: Malignant Rhabdoid tumour of the kidney)

Given the relative rarity of paediatric renal tumours and in particular rare subgroups, it is necessary to recruit as many patients as possible. Over the last decades more than 10.000 children have been prospectively enrolled in SIOP Wilms Tumour studies and trials (Figure 3.2). Since SIOP 93-01 SIOP-RTSG registered nearly 8.000 patients with a renal tumour from 261 centres out of 28 countries. All of them have been treated according to harmonised European trials protocols. This has resulted in more standardised diagnostic procedures, improved risk stratification, and adjusted treatment recommendations for most renal tumours.

The hallmark of the SIOP-RTSG approach is the preoperative chemotherapy (Vincristine and Actinomycin-D in localized and with the addition of Doxorubicin in metastatic disease) without preceding mandatory histological assessment. This has the clear evidence-based benefit of down staging tumours, thereby sparing survivors the late effects of Doxorubicin or radiotherapy by around 20% compared to patients treated with immediate surgery [1]. Nevertheless, this approach carries the risk of misdiagnosis (< 5%), as currently the so-called non-Wilms tumours cannot be identified by standard radiology or biomarker assessment.

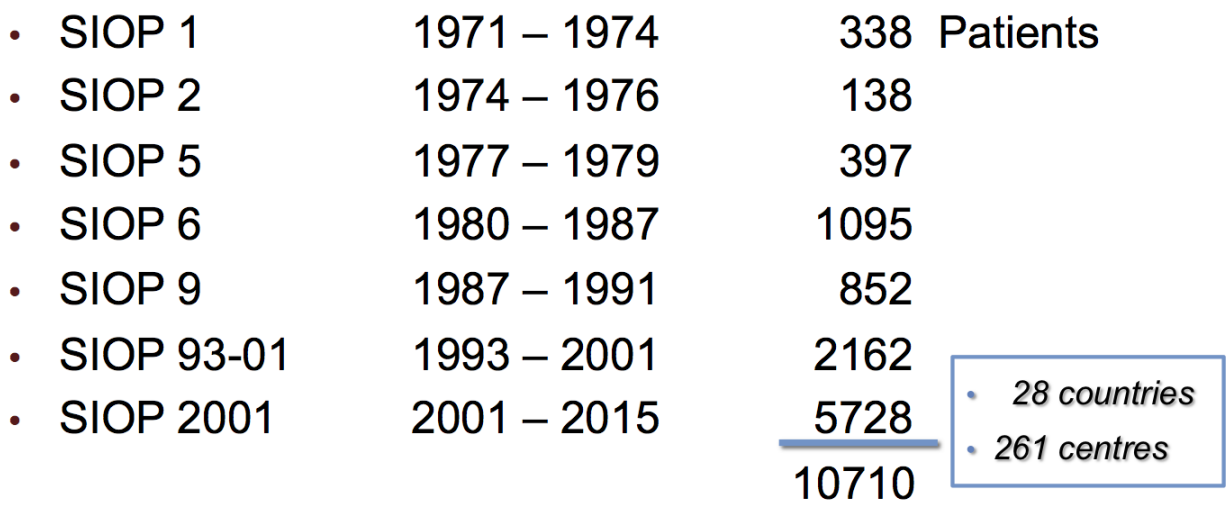

Figure 3.2: Number of patients enrolled in prospective SIOP Wilms Tumour trials.

The current diagnostic, treatment and integrated research UMBRELLA protocol (part A) serves as an entry for including all children with a renal tumour in Europe and other participating centres in SIOP-RTSG. Subsequently, treatment of each participant's renal tumour is recommended according to the SIOP 2015 treatment guidelines, which provides treatment strategies for all WT patients and all children with other renal tumours. These recommendations are mainly based on the results from the previous SIOP and COG (Children's Oncology Group in North America) trials. According to the results of the recently closed SIOP 2001 trial all children with localized stage II and III intermediate risk tumours will receive no Doxorubicin in the postoperative chemotherapy as the new standard of care. The detailed clinical treatment guidelines and follow up protocols for all renal tumours in children and young adults are available to all participating partners and explained in the upcoming UMBRELLA protocol.

The overall aim of the UMBRELLA protocol is to harmonize the clinical relevant standard diagnostic procedures for all paediatric renal tumours within SIOP and to provide imaging studies and biomaterial from all of these patients to find new and better risk factors for treatment stratification and molecular targets for novel therapeutic approaches. This will help to improve short and long term outcomes for all children with renal tumours through the introduction of a more 'personalised' approach. In this respect it is foreseen that patients enrolled in the UMBRELLA protocol will also be enrolled in the research project of CHIC dealing with the Nephroblastoma hypermodel.

3.2 Nephroblastoma clinical scenario and clinical question

The Nephroblastoma clinical scenario is described in detail in D2.2 and the additional deliverable D2.5. A summary of the advanced scenario is provided in figure 5.13 of deliverable D2.2 and an update is given below in figure 3.3 that is identical with figure 3.1 of D2.5. All children with nephroblastoma

receive pre-operative chemotherapy based on imaging studies alone. Around 10% of patients do not respond to pre-operative chemotherapy. For these patients primary surgery would be beneficial. **The hypermodel therefore shall answer the question: Will a given nephroblastoma in a patient respond to pre-operative chemotherapy by tumour shrinkage, yes or no?** After clinical validation of the hypermodel the tool will be used prospectively to guide the initial treatment of children with nephroblastoma.

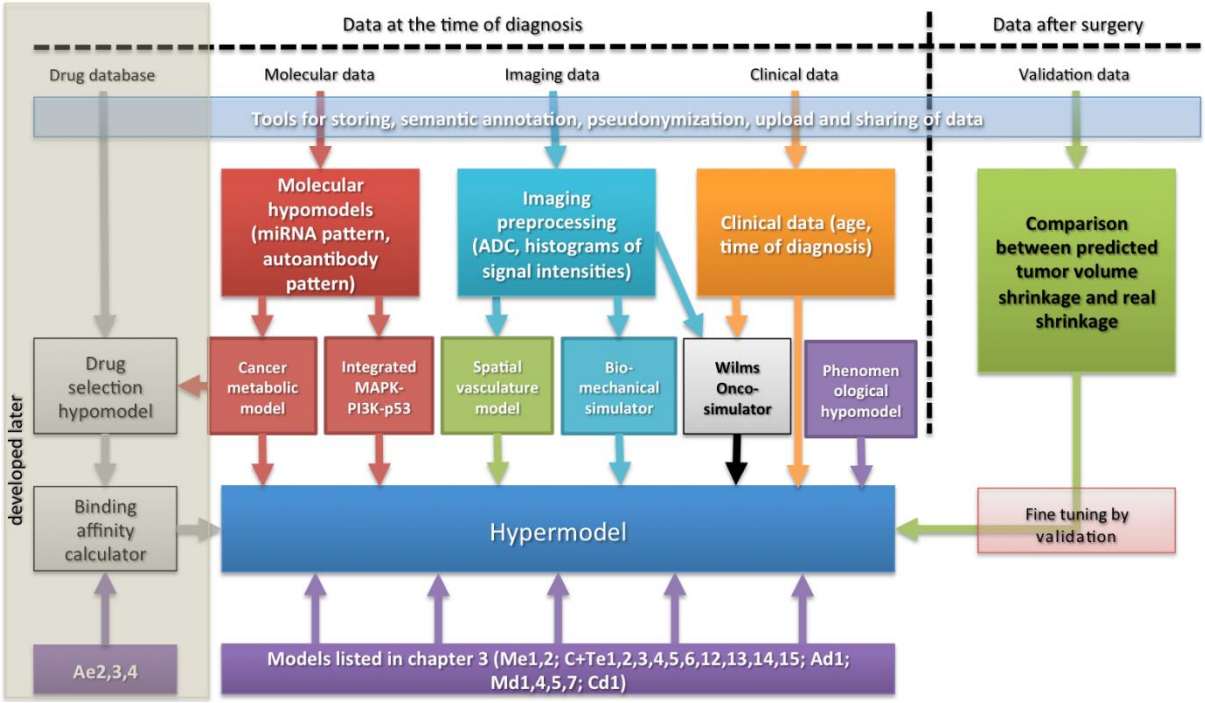

Figure 3.3: Schematic view of the advanced nephroblastoma scenario described by the linkage of hypomodels composing the advanced nephroblastoma hypermodel.

Within the UMBRELLA protocol patients will be enrolled in this scenario and the prediction of the hypermodel will be compared with the real shrinkage of the tumour. Clinical data, segmented image data and miRNA data are uploaded to the CHIC data warehouse. Predicted results of the hypermodel will be stored within a specific CRF of ObTiMA that serves as the UMBRELLA database. This comparison between prediction and reality will be used for validation purposes and fine tuning of the nephroblastoma hypermodel.

3.3 Data

All data that are used within the Nephroblastoma hypermodel are described in detail in D2.2 of the CHIC project. These data are stored in CRFs of ObTiMA that serves as the data management system for the UMBRELLA protocol. A preview of the pathology CRF of the former SIOP 2001 study is shown in figure 3.4. This is compatible with the UMBRELLA pathology CRF.

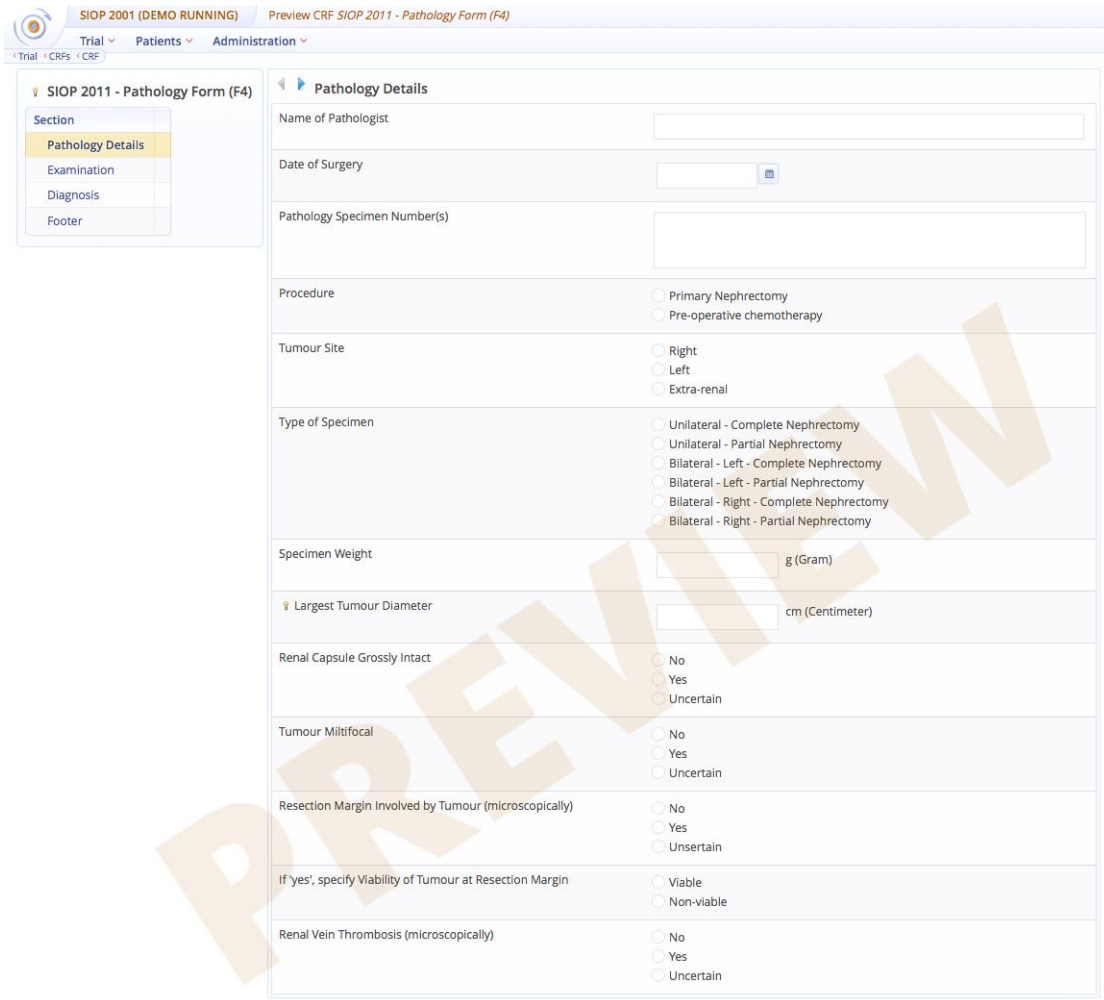

Figure 3.4: Preview of the pathology CRF in SIOP 2001.

3.4 Ethical considerations

An ethical approval for this research will be taken by the Ethical Committee of the 'Ärztekammer des Saarlandes'. As soon as this ethical approval is given it will be provided to the CHIC project. An existing Ethical approval is already available for retrospective data from SIOP 2001. This can be found in deliverable D4.1 (Initial analysis of the ethical and legal requirements for the sharing of data) of the CHIC project.

4 Glioblastoma Multiforme scenario and data

4.1 Background

4.1.1 Glioblastoma Multiforme

High grade gliomas (HGG) are the most common primary tumors in the central nervous system and consist of WHO grade III and WHO grade IV neoplasms: anaplastic/malignant gliomas and glioblastoma multiforme (GBM) respectively. GBM is the most frequent and most malignant of these tumors with a yearly incidence of about 3 - 4 per 100000 adults [2]. The treatment for these patients consists primarily of surgery in order to debulk the tumoral mass for symptomatic relief and to obtain tissue for histological diagnosis, as well as radiochemotherapy to induce optimal local tumor control. The prognosis of patients with GBM remains poor with a median progression free survival (PFS) of only 6.9 months and a median overall survival (OS) of 14.6 months, with a 5-year survival of only 9.8 [3, 4]. Even after maximal treatment with surgery, radiotherapy and chemotherapy, relapse is universal and is believed to be due to the extensive spread of tumor cells into surrounding regions of the brain [5, 6]. As such, there is an obvious need for more effective new therapies that can improve clinical outcome. Currently, preclinical and early clinical research is focused on new alternative approaches, such as more selective therapies targeting intracellular signalling pathways or surface molecules, anti-angiogenesis strategies and immune therapy.

4.1.2 Immune therapy

Immune therapy, and in particular dendritic cell (DC) vaccination, is an emerging treatment modality being explored in preclinical research and clinical trials. In DC vaccination it is aimed to activate the patient's own immune system against the tumor to kill remaining tumor cells. By inducing immunological memory, this could theoretically lead to sustained long-term anti-tumoral protection. Despite the promising effect of DC based immune therapy for HGG, its clinical benefit may be restricted to only a subgroup of patients [7, 8].

4.1.2.1 Principle of immune therapy and tumor vaccination based on dendritic cells

It is hypothesized that there is a delicate balance and interaction between the tumor and the immune system: on the one hand the tumor produces cytokines that suppress the natural tumor-induced immune response by T cells and on the other hand an endogenous antitumor immune response is triggered by the tumorantigens (bottom left side of figure 4.1).

Active specific immune therapy, or therapeutic tumor vaccination, as shown in figure 4.1 targets to disturb this balance in favour of the immune response. The first step is neurosurgery; debulking of the tumor reduces the tumor-induced suppression of the immune system and provides a source of autologous tumor antigens (upper yellow arrow). Induction of an antitumor T cell response requires tumor antigen presentation to the T cell and additional activation signalling. DCs function as antigen presenting cells (APC) and stimulate T cell activation. As local APC in the brain are lacking, DC culturing and *ex vivo* processing can be performed in the lab. Monocytes (M in figure 4.1), harvested from the patient in a single session of leukapheresis (lower yellow arrow), are first differentiated to immature DCs (DCi, d in figure 4.1). After loading these DCi with the autologous HGG-tumor-lysate (HGG-L), they process the tumor antigens and differentiate further to mature DCs (DCm, D in figure 4.1), resulting in DCm-HGG-L. This patient-specific autologous product is then injected intradermally in the patient and when they reach the lymph nodes (purple area) the DCs ought to perform their function as APC and T cell activator. Circulating activated antitumor T cells can pass the blood-brain barrier (BBB) and attack remaining tumor cells (top left side of figure 4.1) which is the aimed result: an antitumor immune

response induced by DC vaccination. After induction of an immune response immunological boosting is aimed for with tumor lysate vaccination (HGG-L).

At cellular level there is a complex interaction between different cell populations: tumor cells, effector T cells, memory T cells, regulatory T cells (Tregs) and macrophages with different functional phenotypes. Most likely all these cells interact with each other and the net result of these complex interactions may well determine the outcome upon immune therapy.

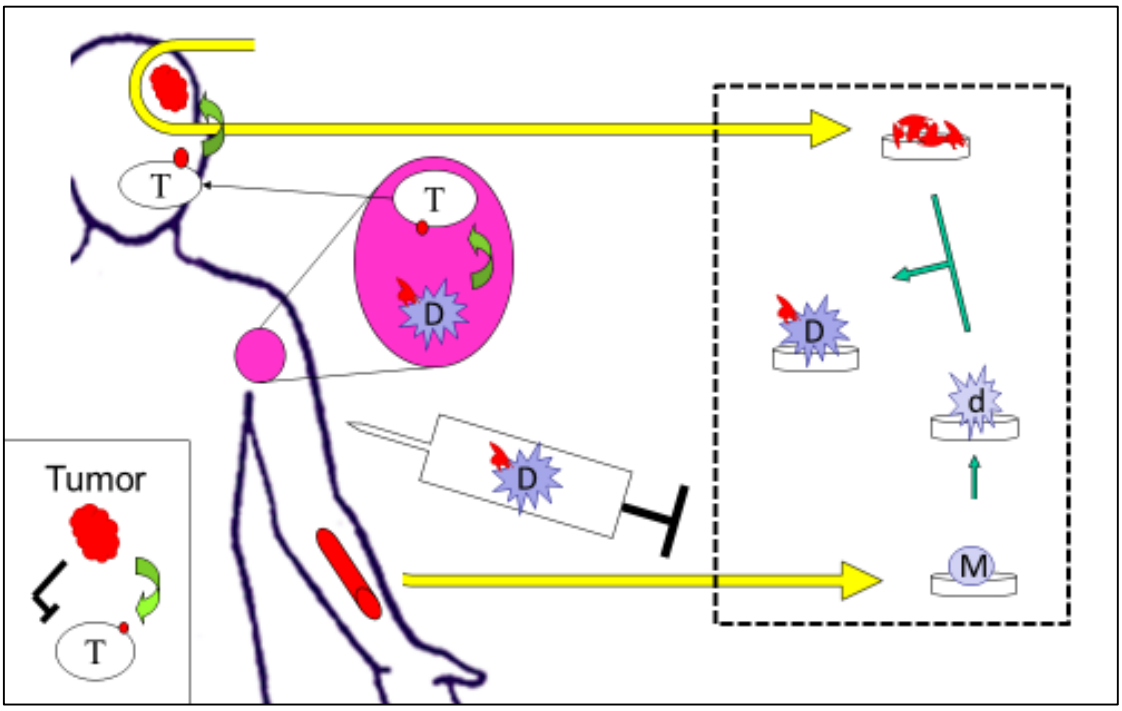

Figure 4.1: The principle of immune therapy: disturbing the balance between the tumor and the immune system to induce an antitumor T cell response by vaccination with cultured DCs loaded with autologous tumor lysate. (T = T cell, M = monocyte, d = immature dendritic cell and D = mature dendritic cell)

4.1.2.2 Immune therapy at UZ Leuven

The HGG-IMMUNO-2003 trials were consecutive clinical trials to treat patients with relapsed HGG with DC vaccination. The sequential protocols in consecutive cohorts of patients with relapsed HGG aimed to prove feasibility and efficacy of immune therapy for HGG and to dissect different aspects of this therapy in order to find a putative ideal vaccination- and treatment strategy. Based on experiences derived from these cohort trials in relapsed patients, strategies of immune therapy were transferred to be integrated within primary multimodal treatment strategies. Based on the results of this phase I/II trial HGG-2006 the HGG-2010 trial was initiated: *"A phase IIb prospective placebo-controlled double blind randomized clinical trial for the treatment of patients with newly diagnosed glioblastoma multiforme with tumor vaccination as "add-on therapy" to standard primary treatment"*. This trial was designed to treat patients with GBM grade IV with a multimodal treatment strategy consisting of surgery, radiochemotherapy, maintenance chemotherapy and immune therapy. Subsequent to the HGG-2006 feasibility- and safety/toxicity-study, the HGG-2010 trial aimed to evaluate the efficacy of immune therapy, integrated as DC vaccination after radiochemotherapy and boost vaccines during maintenance chemotherapy, in a placebo-controlled randomized clinical trial (RCT).

4.1.2.3 Translational research after the HGG-2010 trial

DC vaccination, in the experimental protocol referred to in the former paragraphs has been fully integrated in the postoperative radiochemotherapy and as such final outcome in the HGG-2010 trial inherently reflect mutual and/or additive interactions of surgery, radiotherapy, chemotherapy and immunotherapy. In that concept, translational data regarding pathology, molecular genetics and immune profiles in stored blood samples and diagnostic tumor samples are being examined separately in the Glioma Translat study.

Active immune therapy has shown a potential therapeutic benefit in at least a subset of GBM patients by improvement of the clinical outcome [8]. Identification of this subgroup might shed new light on the potential future of immunotherapy for brain cancer.

4.2 Glioblastoma Multiforme clinical scenario

4.2.1 Definition of the clinical problem – question to CHIC

As described in section 4.1.2.2 a placebo-controlled RCT was conducted at UZ Leuven with integration of immune therapy based on DC vaccination into the primary treatment regimen for patients with newly diagnosed GBM. The clinical course of this trial and the integration of translational research on stored tissue and blood samples is described in detail in section 4.2.2.

During these trial multiscale data were collected for each patient: clinical data, MRI imaging data, biochemical data, pathological data, treatment data and outcome data. These data include known clinically important parameters but also samples for more experimental data collection (e. g. immune monitoring) of which the exact contribution is currently unknown but part of the research question posed in the CHIC environment.

It is already known that DC vaccination is not useful for every GBM patient: a subgroup of patients reaches long term survival, defined as OS >24 months after surgery. From a clinical point of view, it is of much interest to characterise this subgroup and be able to predict which patients are expected to reach long term survival. GBM is probably a more heterogenous group of malignant brain cancer subtypes with different outcomes than classically described. Subtyping of patients, predicting outcome and stratification of patients is a first requirement for personalized medicine in which a more targeted therapy to a specific patient with a specific subtype of GBM can be administered. **Through modelling within the CHIC environment, we want to explore if a patient with certain clinical and pathological characteristics at diagnosis and its specific immune profile is predisposed to become a long-term survivor (or on the contrary is predicted to have early relapse) in the context of a combined postoperative radiochemoimmunotherapy confined to the characteristics of the study population. Moreover, we want to know whether immunotherapy in the form of DC vaccination is helpful to reach this long-term survival and if so, if this vaccination should be given early or late.**

For the first question the predictive value of biological markers related to survival and immune responsiveness needs to be evaluated. The second question evaluate if DC vaccination can initiate or increase anti-tumor immune responses which have a clinical benefit for the patient with GBM. The third question evaluates if immune responses can be better induced early or late in the standard treatment schedule.

4.2.2 Course of clinical practice – clinical scenario

For the GBM scenario the ideal sequence of interventions for the patient in the clinical practice of the placebo-controlled RCT HGG-2010 (with integration of the translational research on stored tissue and blood samples) has been the following: (see figure 4.2)

0) Pre-operative diagnostics

When a high-grade astrocytoma is suspected on pre-operative imaging, the tumor location and the possibility for surgical resection should be assessed. (figure 4.2: DIAGNOSIS)

A complete medical history and clinical examination are performed.

1) Neurosurgery and Pathology

The resection of the tumor aims to bring the tumor burden to a state of minimal residual disease; ideally, the tumor should be macroscopically completely resected. Also, a part of the tumor is provided for pathology and the translational research. Preservation of the tissue as a source of tumor antigens for preparing the vaccines should be taken into account. (figure 4.2: **NEUROSURGERY**)

Postoperative assessment of the extent of resection (EOR) is made at 2 levels: the assessment by the neurosurgeon and a radiological assessment (reference radiology), for which an MRI scan without and with Gadolinium within 72 hours after surgery is mandatory. (figure 4.2: **po-MRI**)

Routine anatomo-pathological examination should histologically confirm the diagnosis of GBM WHO IV and reference pathology for central revision should approve this too. The paraffin embedded tumor sample is stored for genetic and immunohistochemical translational research. (figure 4.2: PATHOLOGY)

2) Inclusion

The patient is included in the trial after verification of inclusion and exclusion criteria and evaluation of all information on the clinical, neurosurgical, radiological and pathological data. *Inter alia* age, Karnofsky performance status (KPS), first diagnosis of histologically proven GBM WHO IV, confirmed diagnosis by reference pathology, EOR, postoperative (MRI) imaging, free from corticosteroids, availability of tissue for immune therapy,… (figure 4.2: INCLUSION)

3) Randomization and Leukapheresis

At time of leukapheresis, randomization is performed with recursive partitioning analysis (RPA) classes as stratification variable. (A concealing, random allocation algorithm was used to organize the randomization.) (figure 4.2: RANDOMIZATION)

Leukapheresis is performed prior to radiochemotherapy and around 1 week after complete withdrawal of pre-operative corticosteroids. This procedure harvests circulating white blood cells (WBC) from which peripheral blood mononuclear cells (PBMC) are purified in the laboratory and which will later be differentiated to DCs. (figure 4.2: **LEUKAPHERESIS**)

At this time point a first sample for hematological monitoring is taken. For this, standard blood analysis (complete formula) is performed. Moreover, PBMCs are isolated and serum is stored for translational research. This time point is of particular interest because blood cells are not yet exposed to radio- or chemotherapy. (figure 4.2: **BLOOD** + **PBMC**)

4) Radiochemotherapy

Radiochemotherapy according to the Stupp protocol [3] is part of the standard treatment for patients with primary diagnosed GBM and thus regardless of randomization. Simultaneous to conventional fractionated irradiation with a total dose of 60Gy, 6 weeks of daily concomitant chemotherapy (TMZr) is administered at a dose of 75mg/m²/d. (figure 4.2: **RADIOCHEMOTHERAPY**)

Clinical, medical, radiological and hematological monitoring is performed at the reference hospital.

5) Immune therapy – Induction phase

Experimental group: Four weekly DCm-HGG-L injections take place. (figure 4.2: **VACCINE 1-4**)

Control group: Four weekly placebo injections take place. (figure 4.2: **PLACEBO 1-4**)

Regardless of randomization:

- At each injection moment data on disability, self-reliance and quality of life (QoL) are recorded by patient self-assessment using the Fertgkeitenskala Münster-Heidelberg (FMH) scale, the KPS scale and the QoL questionnaires EORTC QLQ-C30 v2.0 and BCM20.
- Clinical and medical monitoring is done at each injection. Hematological follow-up takes place at the time of vaccine/placebo 1 and 4. (figure 4.2: **BLOOD**)
- Samples for immune monitoring performed on PBMC and serum are collected at the time of vaccine/placebo 1. (figure 4.2: **PBMC**)
- Radiological follow-up at the reference hospital is planned at the end of the induction phase/at the start of maintenance chemotherapy. (figure 4.2: **MRI**)

6) Maintenance chemotherapy TMZm

Maintenance chemotherapy according to the Stupp protocol [3] is part of the standard treatment for patients with primary diagnosed GBM and thus regardless of randomization. TMZm is administered for up to 6 cycles of 28 days. A standard 5-day schedule is used with a dose of 150mg/m²/d for the first cycle and 200mg/m²/d from the second cycle on, as long as there is no hematological toxic effect. (figure 4.2: TMZm) At day 8 of cycle TMZm I, TMZm II, TMZm III and TMZm VI boosting injections take place (see **7) Immune therapy – Boosting phase**).

Clinical, medical and hematological monitoring is performed at the reference hospital.

Radiological follow-up at the reference hospital is planned around TMZm I, TMZm III and TMZm VI. (figure 4.2: **MRI**)

7) Immune therapy – Boosting phase

Experimental group: At day 8 of cycle TMZm I, TMZm II, TMZm III and TMZm VI immunological boosting with HGG-L injections takes place. Further boosts with HGG-L are injected each 3 months (12 weeks) depending on the amount of HGG-L available. (figure 4.2: **VACCINE 5-9**)

Control group: At day 8 of cycle TMZm I, TMZm II, TMZm III and TMZm VI placebo injections take place. (figure 4.2: **PLACEBO 5-8**)

Regardless of randomization:

- If the TMZm course was postponed (e.g. due to haematological reasons), the injection in the boosting phase was postponed as well.
- At each injection moment data on disability, self-reliance and quality of life are recorded by patient self-assessment using the FMH scale, the KPS scale and the QoL questionnaires EORTC QLQ-C30 v2.0 and BCM20.
- Clinical and medical monitoring is done at each injection. Hematological monitoring takes place at the time of vaccine/placebo 7 and 8 (and 9 in the experimental group). (figure 4.2: **BLOOD**)
- Samples for immune monitoring performed on PBMC and serum are collected at the time of vaccine/placebo 7 and 8. (figure 4.2: **PBMC**)

- Radiological follow-up at the reference hospital is planned around TMZm III and TMZm VI as described in **6) Maintenance chemotherapy TMZm**. (figure 4.2: **MRI**)

8) Unblinding and follow-up

The treatment is cancelled in case of progressive disease/tumor progression. Unblinding of the randomization takes place and new treatment strategies are evaluated at the reference hospital.

In case there is no progressive disease during the course of the standard treatment, the PFS after 6 cycles maintenance chemotherapy (PFS 6m) is determined based on the MRI foreseen after TMZm VI (i.e. the end of the standard treatment) and unblinding takes place. (figure 4.2: **UNBLINDING**)

Experimental group: Further boosts with HGG-L are injected each 3 months (12 weeks) depending on the amount of HGG-L available. (figure 4.2: **VACCINE 9-…**)

At each injection moment data on disability, self-reliance and quality of life are recorded by patient self-assessment using the FMH scale, the KPS scale and the QoL questionnaires EORTC QLQ-C30 v2.0 and BCM20.

Clinical and medical monitoring is done at each injection. Hematological monitoring takes place at the time of vaccine 9. (figure 4.2: **BLOOD**)

Radiological follow-up at the reference hospital is planned at least each 3 months around the time of vaccination. (figure 4.2: **MRI**)

Control group: Active immune therapy is started and follows a similar schedule as in the experimental group: 4 weekly DCm-HGG-L injections, after 4 weeks injections with HGG-L take place every 4 weeks for 3 times and then further each 12 weeks, depending on the amount of HGG-L available and the course of disease. (figure 4.2: **VACCINE 1*-…***)

At each injection moment data on disability, self-reliance and quality of life are recorded by patient self-assessment using the FMH scale, the KPS scale and the QoL questionnaires EORTC QLQ-C30 v2.0 and BCM20.

Clinical and medical monitoring is done at each injection. Hematological monitoring takes place at the time of vaccine 4*, 7*, 8* and 9*. (figure 4.2: **BLOOD**)

Radiological follow-up at the reference hospital is planned at least each 3 months around the time of vaccination. (figure 4.2: **MRI**)

Week					
0	DIAGNOSIS				
	NEUROSURGERY + po-MRI + PATHOLOGY				
2	INCLUSION				
	RANDOMIZATION + LEUKAPHERESIS + BLOOD + PBMC				
4		RADIO CHEMO THERAPY		RADIO CHEMO THERAPY	
6					
8					
	BLOOD + PBMC	PLACEBO 1		VACCINE 1	BLOOD + PBMC
10		PLACEBO 2		VACCINE 2	
		PLACEBO 3		VACCINE 3	
12	BLOOD	PLACEBO 4		VACCINE 4	BLOOD
	MRI	TMZm I		TMZm I	MRI
14		PLACEBO 5		VACCINE 5	
16					
		TMZm II		TMZm II	
18		PLACEBO 6		VACCINE 6	
20					
	MRI	TMZm III		TMZm III	MRI
22	BLOOD + PBMC	PLACEBO 7		VACCINE 7	BLOOD + PBMC
24					
		TMZm IV		TMZm IV	
26					
28					
		TMZm V		TMZm V	
30					
32					
		TMZm VI		TMZm VI	
34	BLOOD + PBMC	PLACEBO 8		VACCINE 8	BLOOD + PBMC
	MRI	UNBLINDING			MRI
36					
		VACCINE 1*			
38		VACCINE 2*			
		VACCINE 3*			
40	BLOOD + MRI	VACCINE 4*			
42					
44		VACCINE 5*			
46				VACCINE 9	BLOOD + MRI
48		VACCINE 6*			
50					
52	BLOOD + MRI	VACCINE 7*			

Figure 4.2: Outline of ideal sequence of interventions in the clinical practice of the performed RCT.

4.3 Generated data used in the GBM scenario

All the above mentioned elements of the GBM scenario provide data that will be made available to compose the hypo- and hypermodels.

4.3.1 Data source

The data for the GBM scenario originate from the finalized RCT HGG-2010 and the translational research protocol Glioma Translat (section 4.1.2.2 and 4.1.2.3). Data on the interventional therapy (DC vaccination as “add-on” treatment) and the translational research are generated at UZ Leuven and KU Leuven, data on standard treatment at reference hospitals.

Because the data were generated during and in the context of this trial and research (and not specifically for CHIC), not all data sets can provide *all* elements of the scenario described above. Also, retrospectively trying to collect data for the GBM scenario resulted in data sets with missing data, especially for patients from reference hospitals. Moreover, reference hospitals with other accreditations can provide other absolute values (e.g. for blood counts).

In general, all sort of deviations can occur due to the predominance of the patient’s treatment and well-being at all time. The amount and availability of all data is always a results of the clinical reality and the patient-specific course of disease, explaining the strong variation of extensiveness of the data sets.

4.3.2 Data storage

Most data were generated at the several reference hospitals where patients participating in the RCT had their standard therapy and general follow-up (as listed in section 4.2.2). During and after the lifetime of the trial these trial data are collected and filed as source documents at the local study centre UZ Leuven (figure 4.3), where they were joined with the trial-specific data.

During the lifetime of the CHIC project the clinical scenario’s data were entered in the project’s clinical trial management system ObTiMA (Ontology based Trial Management Application), the web-based GCP compliant data management system provided by USAAR. This project-compliant data management system will connect to the CHIC repositories to upload the data directly (figure 4.3).

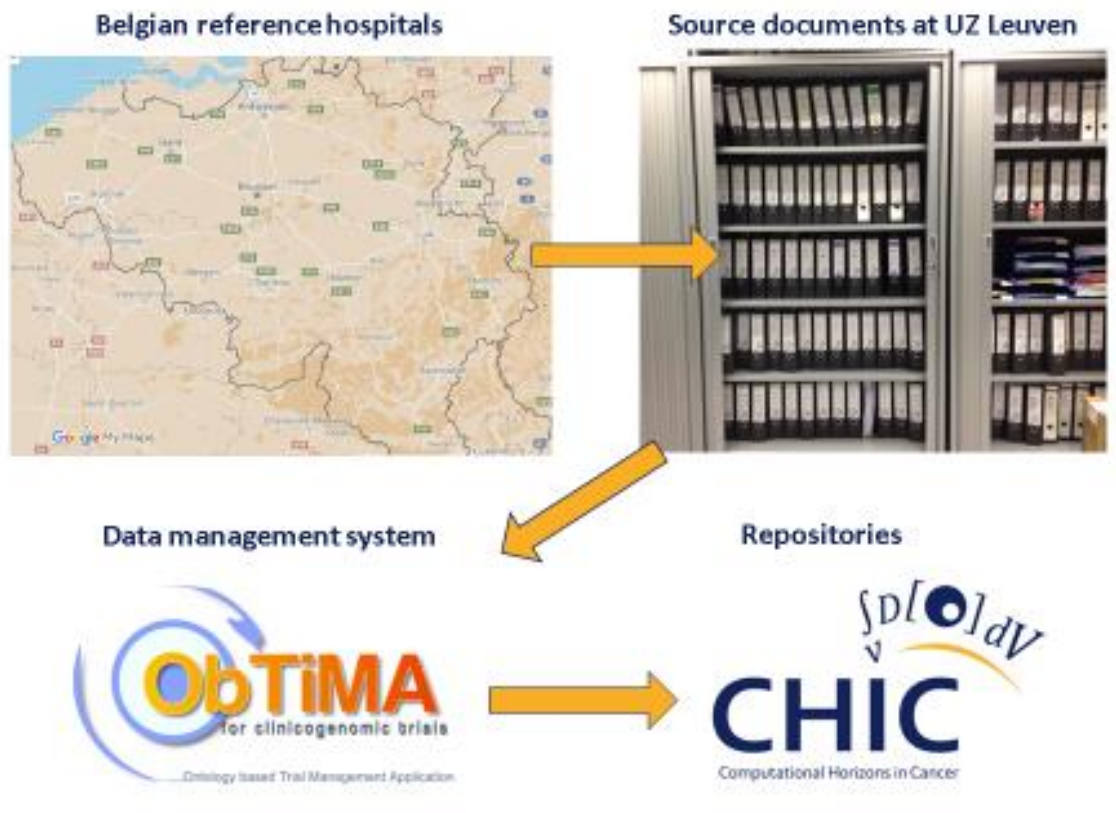

Figure 4.3: The data from Belgian reference hospitals are collected at the trial centre at UZ Leuven. Trial and research data are stored in the data management system and transferred to the CHIC repositories.

All data collected at time of diagnosis, during treatment, at follow-up and data resulting from the translational research are entered in ObTiMA by KU Leuven members.

- To be able to store and manage these GBM scenario specific data a new set of specific case report forms (CRF) is created. (figure 4.4.1)
- For each patient a record is created and for every study event in the clinical course of the specific patient the according CRFs are coupled and repeated if necessary (e.g. Patient Blood Counts). (figure 4.4.2)
- The data were entered in the CRFs as complete as possible, based on the availability of trial-specific (UZ Leuven) and non-specific (reference hospitals) data in the source documents. (figure 4.4.3)

MRI images are stored as DICOM files in the PACS at UZ Leuven and can be uploaded directly to the repositories after processing with an on-site download/anonymization tool.

Acronym	Name	Version	Description
	Adverse events	1.0019	Adverse events during trial
	Baseline Patient Characteristics	1.0153	Patient information Course of disease
	Baseline Patient Characteristics	1.0193	Patient information Course of disease
	Chemotherapy	1.0009	Adjuvant chemotherapy
	Immunotherapy	1.0163	Leukapheresis Dendritic cell quality Lysate vaccines
	Medication	1.0059	Relevant medication during trial
	Patient Blood Counts	1.0081	Blood values during trial
	Patient Blood Counts Female	1.0092	Blood values during trial
	Patient Questionnaire	2.0001	Fertigkeitenskala Münster-Heidelberg Karnofski Performance Scale EORTC QLQ-C30 BCM20
	Peri-operative radiology	1.0051	Pre-operative imaging Post-operative MRI
	Radiochemotherapy	1.0040	Radiotherapy Concomitant chemotherapy
	Trial radiology	1.0018	Imaging during the clinical trial
	Vaccination	1.0033	Vaccination information

Figure 4.4.1: Newly created set of GBM specific CRFs.

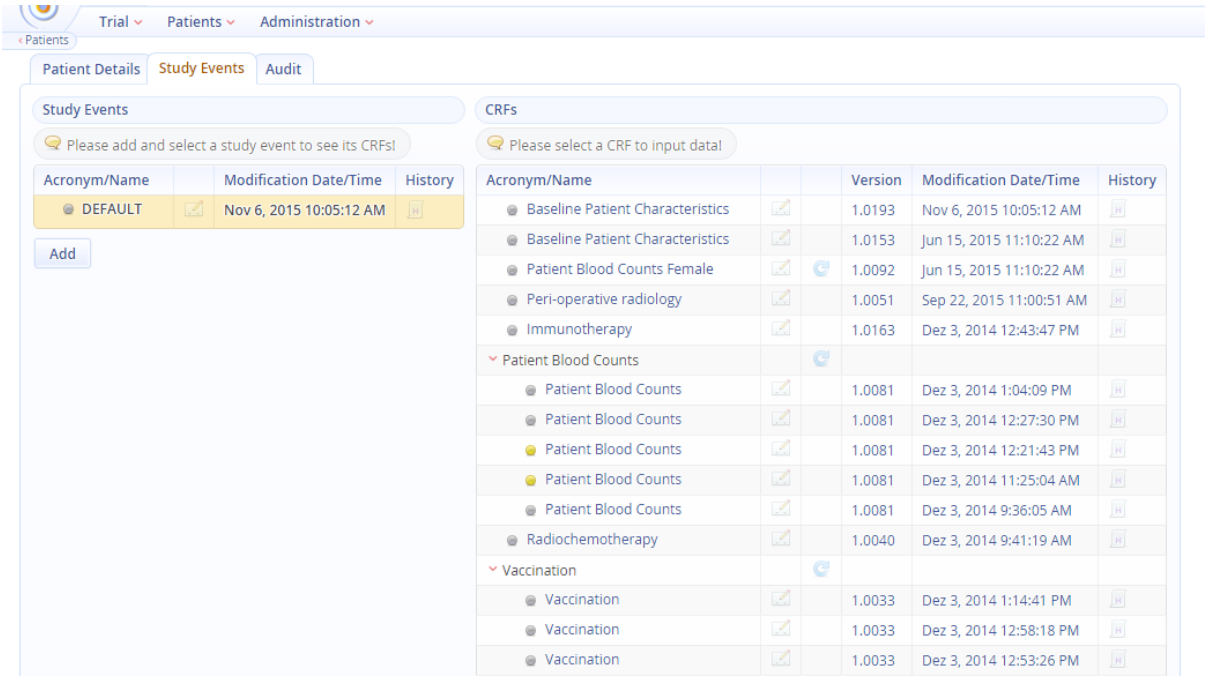

Figure 4.4.2: CRFs are coupled to the patient record according to the clinical course.

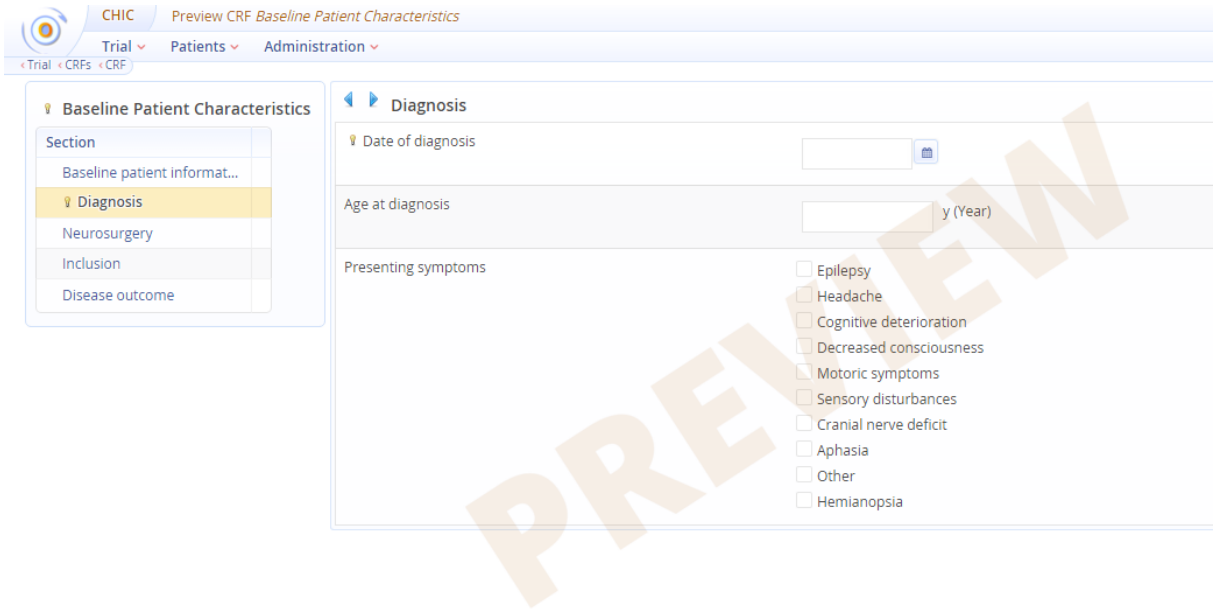

Figure 4.4.3: Example of a CRF to be completed with data from the source documents.

4.3.3 Data description

The data will be described as they are presented in the CRFs of the data management system ObTiMA. Reference is made to the scenario elements as presented in section 4.2.2 and figure 4.2.

4.3.3.1 Baseline Patient Characteristics (version 1.0193)

This CRF contains data generated at time of DIAGNOSIS, INCLUSION, **NEUROSURGERY**, **po-MRI** and UNBLINDING.

- The baseline patient characteristics (sex, patient history and relevant medication), characteristics at diagnosis (age and presenting symptoms) and at inclusion (KPS, MMSE and the derived RPA) feature the patient and form the basis of personalized medicine. No missing data should occur.
- RPA classification subdivides patients with HGG in 3 categories of clinical risk-profile based on the pre-treatment variables age, pathology, self-reliance (KPS) and mental state (MMSE). In the case of GBM, RPA 3, RPA 4 or RPA 5 are possible, with a better prognosis in lower classes. The HGG-2006 trial demonstrated that these pre-treatment variables are relevant in studies on DC vaccination as an important predictor for PFS as well for OS [9].

RPA class	Age	Pathology	KPS	MMSE
III	<50	GBM	90-100	
IV	<50	GBM	<90	
IV	≥50	GBM	70-100	≥27
V	≥50	GBM	70-100	<27

Table 4.1: Determination table for RPA classification for GBM patients.

- Extent of resection (EOR) is an independent risk factor: a more extensive resection is a prognostic parameter for better outcome; ideally, a gross total resection is performed.

  Neurosurgical assessment can result in

  - Total resection, no macroscopic tumor tissue left
  - Subtotal resection: residual tumor < 2cm³, or only visible local tumor infiltration
  - Partial resection: residual tumor > 2cm³
  - Biopsy
  - Not specified: post-operative MRI > 72h
- All these data are available at diagnosis/the beginning of standard treatment and thus are the first input for the predicting model.
- Definition of disease progression (PFS after 6 cycles TMZm and PFS data of event) is based on the review of longitudinal MRI imaging series. The RANO criteria are used to define the progression status.
- The outcome parameter OS drives the clinical question. If this parameter is not entered when the data set is made available to the consortium, the critical endpoint is reached and the patient reached long-term survival (OS > 24 months). Thus, missing data for OS indicate long-term survival.
- Also the further course of disease (described in CRF section 'Disease outcome' as salvage therapy) influences the long-term survival but doesn't have a predictive value in our clinical question.

4.3.3.2 Peri-operative radiology

This CRF contains data generated at time of **DIAGNOSIS** and **po-MRI**.

The pre- and postoperative MRI are annotated with categorical values (volume, contrast enhancement, perfusion, diffusion and oedema) by a qualified radiologist.

- No specific localisation is known to be a predictive factor, but localisation has an indirect effect on outcome as deep or eloquent located tumors are not suitable for resection. A more extensive lesion is in general more difficult to resect, and represents a worse prognosis.

  Moreover, the 6 molecular subtypes as described by Sturm and Pfister [10] of GBM tumors are related with their location and age at diagnosis.
- The prognostic parameter EOR has particular importance in the case of DC vaccination as it lowers the need for corticosteroids and diminishes the immunosuppressive properties of the tumor (as theoretically described in section 4.1.2.1).
- Radiological assessment by the radiology reference centre can result in
  - No visible tumor, no contrast enhancement (RTV = 0)
  - Linear contrast enhancement at the border of the resection cavity or nodule (RTV < 2cm³)
  - Residual tumor as nodular lesion, measurable in two dimensions (RTV > 2cm³)
  - No obvious change to the pre-operative imaging

- Partly contrast-enhancing GBMs most likely present secondary GBM (i.e. a GBM arising from a less malignant lesion) and those lesions have a better prognosis. The more contrast - enhancing the tumor, the worse the prognosis.
- The relative regional cerebral blood volume (rrCBV) can be measured with the perfusion MRI imaging technique. The lower the rrCBV, the better the survival; cut-off is rrCBV 1.75.
- The apparent diffusion coefficient (ADC) is a parameter from diffusion MRI, reflecting the cellularity. A higher cellularity of the tumor means a more aggressive tumor and a worse outcome.
- Less oedema is a better outcome, also because it lowers the need for corticosteroids.

4.3.3.3 Radiochemotherapy

This CRF contains data generated at time of **RADIOCHEMOTHERAPY**.

- This treatment modality is part of standard therapy. Unless in case of progressive disease no missing data should occur.
- The dose of TMZr can change during radiochemotherapy because of changes in body weight; dose 2 and 3 are therefore not mandatory.

4.3.3.4 Immunotherapy

This CRF contains data generated at time of **LEUKAPHERESIS**, **VACCINE 1-4** and **VACCINE 1*-4***.

- These data describe the product quality parameters of the autologous elements of the immunotherapy: the composition of the leukapheresis collection, the cell counts and viability during the culturing of the DCs, purity and potency analysis of the DCm-HGG-L by FACS and whole tumor lysate concentration.
- A measure of the purity of the leukapheresis collection product is given. This influences the production of the DC vaccines.
- Technical details of the DCm-HGG-L preparation are fixed, only some variable parameters per patient per vaccine are provided. These parameters during cell culture influence the end product DCm-HGG-L:
    - Monocyte adherence: amount of PBMCs adhered at day 0 of cell culture procedure
    - Number of viable immature DCs: amount of viable immature dendritic cells at day 6 of cell culture procedure
    - Viability of immature DCs: Amount of viable immature dendritic cells to the total amount of cells counted at day 6 of cell culture procedure
    - Number of viable mature DCs: Amount of viable mature dendritic cells loaded with tumor proteins at day 7 of cell culture procedure
    - Viability of mature DCs: Amount of viable mature dendritic cells loaded with tumor proteins to the total amount of cells counted at day 7 of cell culture procedure
- The purity of each of the 4 DCm-HGG-L vaccines is determined by FACS analysis. The relative count of different cell populations on the total cell population in the vaccine is determined; a higher percentage reflects a higher amount of that specific cell population in that vaccine and thus less DCs.

  The following stainings on the total cell population are performed:

- CD3+ population (i.e. T-cells)
- CD56+ population (i.e. NK-cells)
- CD3+/CD56+ population (i.e. NKT-cells)
- CD19+ population (i.e. B-cells)
- CD14+ population (i.e. monocytes, the cells from which DCs are differentiated): here a higher percentage reflects less differentiation of the precursor cells to DCs.
- CD83-CD14-CD11c+ population (i.e. DCi): here a higher percentage reflects less maturation to DCm.
- CD83+ population (i.e. DCm)

Also the potency of the 4 DCm-HGG-L vaccines is determined by FACS analysis. The relative count of specific cell populations is determined in the myeloid cell gate of the vaccine; a higher percentage reflects a higher maturation (CD83 and CD86) or activation (HLA-DR) grade of the DCs in the vaccine.

The following stainings on the myeloid cell gate are performed:

- CD11c+ CD83+ population (during differentiation from DCi to DCm the activation marker CD83 is upregulated)
- CD11c+ CD86+ population (upon maturation the expression of the co-receptor molecule CD86 is upregulated)
- CD11c+ HLA-DR+ population (the expression of the co-stimulatory molecule HLA-DR is upregulated in response to stimulation)

- The total amount of proteins in the whole tumor lysate and total volume available after tumor processing determine how many vaccines a patient theoretically can receive.

4.3.3.5 Vaccination

This CRF contains data generated at time of **RANDOMIZATION**, **VACCINE 1-…**, **VACCINE 1*-…*** and **PLACEBO 1-…**.

- The amount of cells or lysate injected may influence the immune responsiveness. In case of missing data/CRF, the patient probably never received immune therapy due to early relapse.
- Glucocorticoid administration, clinically needed for peritumoral oedema, has a negative effect on DCs and is unwanted when treating with DC vaccination.

4.3.3.6 Patient Questionnaire

This CRF contains data generated at time points depicted with **VACCINE 1-…**, **VACCINE 1*-…*** and **PLACEBO 1-…**.

- These self-assessment questionnaires are composed of standardized lists and processed with standardized scoring calculations.
- Fertgkeitenskala Münster-Heidelberg (FMH) is a questionnaire for self-assessment of disability with six function groups: locomotion, eating and drinking, body care, communication, reading/writing/calculating, and general independence. These functions are evaluated using a total of 56 items to be answered with “yes” or “no.” The “yes” answers are counted and

transformed to an age-dependent percentile using a published table of normal values. A score of 50% or more is considered as normal.

- The Karnofsky Performance Status (KPS) scale measures self-reliance and runs from 100 to 0, where 100 is "perfect" health and 0 is death. The KPS allows patients to be classified as to their functional impairment.
- The EORTC quality of life questionnaire (QLQ-C30)) is an integrated system for assessing the health related QoL of cancer patients participating in clinical trials. It incorporates five functional scales (physical, role, cognitive, emotional, and social), three symptom scales (fatigue, pain, and nausea and vomiting), a global health status / QoL scale, and a number of single items assessing additional symptoms commonly reported by cancer patients (dyspnea, loss of appetite, insomnia, constipation and diarrhea) and perceived financial impact of the disease.
- The Brain Cancer Module (BCM20) is a supplemental questionnaire developed specifically for use with general questionnaires such as the QLQ-C30 in patients with brain cancer. It contains 4 multi-item scales (future uncertainty, visual disorder, motor dysfunction and communication deficit) and 7 single items (headache, seizures, drowsiness, hair loss, itching, weakness in the legs and bladder control).

4.3.3.7 Chemotherapy

This CRF contains data generated at time of **TMZm**.

- This treatment modality is part of standard therapy.
- The treatment schedule and doses (/m²) are fixed as long as there is no hematological toxic effect, then postponing the cycle or reducing the dose is possible.

4.3.3.8 Trial radiology

This CRF contains data generated at time points depicted with **MRI**: this is possible during **RADIOCHEMOTHERAPY** and **TMZm**, at **PLACEBO 4**, **PLACEBO 7**, **PLACEBO 8**, **VACCINE 1**, **VACCINE 4/4***, **VACCINE 7/7***, **VACCINE 8/8***, **VACCINE 9/9***, follow up every 3 months afterwards or for clinical need.

- MRI images are taken at scheduled time points and should be annotated with categorical values (volume, contrast enhancement, perfusion, diffusion and oedema) by a qualified radiologist afterwards. Missing data are likely to occur.

  Imaging at other timepoints is possible due to clinical need, different follow up schedule at reference hospitals or participating in a longitudinal imaging study.
- The routine MRI protocol consists of conventional MRI such as T1-weighted images before and after contrast administration, T2-weighted images and FLAIR-images. These imaging techniques provide structural information on the brain and associated pathology: to determine the location of the pathology, the extent of the lesions and its relationship to the surrounding structures. Moreover, the protocol is further elaborated with two functional techniques: diffusion weighted imaging and dynamic susceptibility weighted imaging, which provide information on cellularity and tissue perfusion, respectively. (See also D2.2)
- The volume of the contrast-enhancement and the volume of perilesional oedema is assessed with manual delineation. The less contract enhancement the better.

Pseudoprogression is a transient contrast enhancement, which suggests tumor progression, but is in reality a reaction towards the provided radiochemotherapy. Pseudoprogression is a marker of tumor sensitivity to the treatment. From a clinical point, the distinction between pseudoprogression and true progression is not always clear. Sometimes a new MRI is performed more early than the standard 3 months, or an additional nuclear imaging examination is done (PET scan).

- Cerebral blood volume in the contrast-enhancing lesions is derived from gamma variate fitting procedure after correction for leakage and circulation.

  There is preliminary evidence that rrCBV values of tumor at the time of diagnosis correlates with overall survival. If rrCBV is above the cut-off, there is tumor progression. A rrCBV below the cut-off is explained by therapy related changes. The higher the rrCBV values, the worse the prognosis.

- Mean diffusivity in the contrast-enhancing lesions (derived from diffusion weighted imaging) is derived from a mono-exponential fitting procedure.

  The ADC is a parameter from diffusion MRI, reflecting the cellularity. A higher cellularity of the tumor means a more aggressive tumor and a worse outcome.

- Oedema is specifically monitored because glucocorticoid administration, clinically needed for peritumoral oedema, has a negative effect on DCs and is unwanted when treating with DC vaccination. The less oedema, the better.

4.3.3.9 Patient Blood Counts – Patient Blood Counts Female

This CRF contains data generated at time points depicted with **BLOOD**: this is possible at **LEUKAPHERESIS**, **PLACEBO 1**, **PLACEBO 4**, **PLACEBO 7**, **PLACEBO 8**, **VACCINE 1**, **VACCINE 4/4***, **VACCINE 7/7***, **VACCINE 8/8*** and **VACCINE 9/9*** at the trail center UZ Leuven and at **RADIOCHEMOTHERAPY** and **TMZm** at the reference hospital.

- Blood samples for standard cell counts are taken at time of leukapheresis as a reference point for further analysis of the biochemical data during treatment.
- Blood samples during radiochemotherapy and maintenance chemotherapy are useful for monitoring the haematological toxic effects of these treatments. Due to different accreditation between the different reference hospitals, missing data will occur for these blood values.
- Standard WBC counts and their differentiation (neutrophils, lymphocytes, etc.) can be of importance. First, these basic blood counts provide absolute numbers of circulating cells, and absolute counts cannot be measured with FACS (cfr. PBMCs). And influence of absolute cell counts on effects of immune therapy can be expected. Secondly, it has already been shown for other cancers that lower pre-treatment lymphocyte counts have a negative prognostic impact.

4.3.3.10 Medication

This CRF contains data that can be generated continuously during standard treatment and immune therapy: during **VACCINE 1-…**, **VACCINE 1*-…*** and **PLACEBO 1-…** at UZ Leuven or during **RADIOCHEMOTHERAPY** and **TMZm** at the reference hospital.

- Medication that interacts with the immune system are of interest.

4.3.3.11 Pathology

This CRF contains data generated by **PATHOLOGY**. This CRF is not available yet due to ongoing translational research on stored samples. As pathological samples are by definition collected at the moment of diagnosis, tumor pathology data provide an important first input on a prediction model.

The available samples are investigated in following ways:

- Genetic analysis is performed to subclassify tumors. It is known from the clinic that not all (histologically proven) GBM behave the same. It has been postulated that GBM are probably a more heterogeneous group of tumors with different genetic profiles. GBM have been classified in 6 subgroups on the base of DNA methylation patterns [10]. We also aim to examine the stored tumor tissue on DNA methylation patterns, in order to correlate clinical results with genetic disturbances.
- On remaining paraffin embedded tumor tissue, immunohistochemistry (IHC)is performed to check the presence, location and phenotype of infiltrating immune cells in the tumor microenvironment. Sections from tumor tissue are stained for CD68 (macrophages), HLA-DR (M1 polarization), and CD163 (M2 polarization). The lymphocytes are stained with CD3 and PD-1. Tumor cells will possibly be stained for Galectin-1 [11] and PDL-1 [12]. This will lead to a better immunological characterization of the tumor tissue, and the interactions of these findings with the systemic immune profiles and general outcome of the patients can then be investigated.

4.3.3.12 Immune monitoring

This CRF contains data generated at time points depicted with **PBMC**: this is possible at **LEUKAPHERESIS**, **PLACEBO 1**, **PLACEBO 7**, **PLACEBO 8**, **VACCINE 1**, **VACCINE 7** and **VACCINE 8**. This CRF is not available yet due to ongoing translational research on stored blood samples. Blood samples for PBMCs and serum were taken at time of leukapheresis as a reference point, and at regular intervals during treatment/follow-up.

The available samples are investigated in following ways:

- PBMCs are thawed and stained with fluorochrome-labeled antibodies to sort cells in different cell populations and analyze activation/suppression markers on these samples with flow cytometry (FACS):
    - T cells will be defined as CD45+CD3+ and subdivided in CD4+ T-helper cells and CD8+ cytotoxic T cells, and further analyzed for expression of PD-1 and CD69. PD-1 and CD69 are markers for more suppressed T cells.
    - Tregs will be defined as CD45+CD3+CD4+CD25+CD127dim and analyzed for expression of PD-1, CD69 and HLA-DR. HLA-DR+ Tregs are a subpopulation of Tregs that have been described to be more immunosuppressive. PD-1 is a surface molecule involved in suppression of these cells.
    - Myeloid cells will be defined as CD45+CD11b+ cells and subdivided in HLA-DR- suppressor cells that can be monocytic (CD15+) or granulocytic (CD14+).

Conventional T cells	Regulatory T cells	Myeloid Derived Suppressor Cells (MDSC)
CD45	CD45	CD45
CD3 CD16/56	CD3	CD11b
CD4	CD4	HLA-DR
CD8	CD25	CD14
CD69	CD127	CD15
PD-1	CD69	
	HLA-DR	
	CD152	
	PD-1	

Table 4.2: Overview of the different PBMC stainings for T cells, Tregs and MDSC.

- Serum was stored at the same moments as PBMCs were isolated. This serum is analyzed with the Cytometric Bead Assay (CBA) technique for MCP-1, IL-10, IL-12, VEGF and IFN-γ. Furthermore, with the ELISA technique Galectin-1 levels is measured.

In literature, peripheral immune responses are reported in 50% of treated patients, but relations with outcome are less clear. In our translational study, we aim to combine basic blood cell counts, PBMC analysis and serum analysis to form a more expanded multi-parameter immune monitoring. This could detect more subtle changes in the immune profile or interactions between parameters, both of which can be more important for correlation with clinical outcome than the study of each parameter on its own.

4.4 Ethical and legal considerations

Most of the data of the GBM scenario were generated in the context of the previously performed and finalized HGG-2010 trial *"A phase IIb prospective placebo-controlled double blind randomized clinical trial for the treatment of patients with newly diagnosed glioblastoma multiforme with tumor vaccination as "add-on therapy" to standard primary treatment" EudraCT 2009-018228-14*, approved by the *Commissie Medische Ethiek UZ KU Leuven* on March 1st, 2010.

An informed consent was obtained for all 136 patients which states that the data will be used for scientific purposes, except for data that will identify the patient.

An extra approval for sharing the data in the CHIC consortium was given by the *Commissie Medische Ethiek UZ KU Leuven* on April 7th, 2014.

The clinical trial was finalized in December 2014; this means that the trial's target value of interest is known at the time of use by the modellers so that the glioblastoma scenario can be entirely processed with de-identified/anonymized retrospective data during the scope of CHIC.

The remaining translational research data were collected in a retrospective analysis research protocol Glioma Translat *"Evaluation of the anatomopathological and molecular genetic contexture in glioblastoma patients treated with surgery and radiochemoimmunotherapy."*, which was approved by the *Commissie Medische Ethiek UZ KU Leuven* on February 2nd, 2016.

KU Leuven also signed the *CHIC Data Provider Agreement* on May 23th 2014 (copy signed by president of CDP was sent back on June 26th 2014) and by this means data protection agreements for sharing data are fulfilled.

Access to the patient data (on site and in ObTiMA) is restricted to the UZ Leuven hospital's trial clinicians and assistants. Access to the data in the CHIC repositories is restricted by the Data Protection Agreements under supervision of the CHIC Center for Data Protection (CDP).

5 Non-small cell lung cancer scenario and data

5.1 Introduction and clinical question

Patients with non-small cell lung cancer (NSCLC) are diagnosed and treated according to national guidelines. This means that patients up to UICC stage IIIa are usually treated by surgery, while patients at UICC stage IIIb and IV are treated by radiochemotherapy. If an adenocarcinoma immunophenotype is verified, at least the patients at inoperable stages qualify for further molecular genetic analysis of their tumor tissue. In Europe it can be expected that about 13-15% of the adenocarcinoma patients carry such mutations. Treatable molecular genetic mutations can occur in Exon 19 and 21 of the EGFR gene, in the B-RAF and ALK gene.
For prognostic purposes adenocarcinomas of the lung can be further subdivided by using the International Association for the Study of Lung cancer/Union for International Cancer Control (IASCL/ UICC) grading system, which means that the prognosis of adenocarcinomas of the lepidic type (Grading: G1) varies considerably from the adenocarcinomas of the acinar type (Grading: G2) and pulmonary adenocarcinomas of the solid type (Grading: G3).

The study set-up of this part of the CHIC project focusses on the analysis of G2 and G3 adenocarcinomas of the lung which are resectable (UICC stage up to IIIa). In this clinical situation it is often very important to determine if adjuvant radio- and/or chemotherapy is necessary to be performed, even when there is a R0-resection and/or a L0 status (lack of lymphatic invasion) or V0 status (lack of blood vessel invasion). The resected tumor specimen allows thorough histomorphological and immunohistological analyses (e.g. Ki-67) and a precise determination of the amount of tumor necrosis. Also, the tissue samples can serve for molecular analyses of the tumor and can be compared with non-neoplastic lung tissue. The use of follow-up imaging of the thoracic organs, i.e. the residual lung parenchyma by computed tomography (CT), will allow to determine the time to progression of the disease and/or define long-term survivors.

By modelling within the CHIC environment, we want to explore if a patient with certain clinical and pathological characteristics at the time of diagnosis and surgical resection of the tumor tissue might show characteristic tumor parameters enabling to predict the likelihood of recurrence and/or metastasis of the tumor or might qualify for clinical surveillance if a relapse is unlikely. Moreover, we want to know whether it can be predicted, if personalized, tumor mutation-specific, therapies can be helpful to prolong the time to recurrence and long-term survival.

5.2 The lung adenocarcinoma clinical scenario

The diagnosis of a pulmonary adenocarcinoma is usually achieved by evaluation of clinical symptoms (persistent cough, hemorrhagic sputum, unexplained or persistent pulmonary infection, analysis of anamnestic factors etc.). After clinical investigation of the patient an imaging procedure is usually performed. The X-ray analysis of the thoracic organs by standard chest X-ray procedures is most often followed by CT scans of both lungs and all other intrathoracic organs. If the findings are suggestive for an intrapulmonary mass, i.e. for a malignant pulmonary tumor, a histological evaluation of the mass is indicated. Afterwards a positron emission tomography (PET) scan or PET/CT scan can be performed especially in order to get information of additional tumor manifestations, either intrapulmonary or in hilar or mediastinal lymph nodes. PET/CT will also give information if a metastatic disease with hematogenous metastasis is likely or not. The tissue diagnosis of malignant lung tumors can be reached, if tumor tissue can be visualized during a bronchoscopy and if tumor tissue can be harvested with a small forceps that can be introduced into the bronchoscope. If no tumor tissue can be taken by bronchoscopy, a transthoracic fine needle biopsy can be performed to get access to tumor tissue. In this case a tissue cylinder will be taken out of the tumor and will be analyzed by histopathological

methods. If the diagnosis of a pulmonary adenocarcinoma or a not otherwise specified non-small cell lung cancer (NSCLC) is made, the case is usually selected for a presentation in a multidisciplinary tumor board. If the board specialists agree that there is a limited disease, a tumor of maximum stage IIIa according to UICC/ TNM classification and no contraindications or significant co-morbidity from other neoplastic or non-neoplastic diseases, then a surgical procedure to remove the tumor from the lung (i.e. by lobectomy) and removal of the regional lymph nodes is recommended. If the patient agrees to this procedure, the surgical removal of the lung/parts of the lung is planned and usually performed within a few days. The lung resection specimen will be transferred to the Institute of Pathology as a native sample in order to perform a frozen section diagnosis or to collect native and standardized formalin-fixed tissue for histopathological tumor typing and staging, resulting in tumor diagnosis.

5.3 Data collection and storage

During the evaluation procedure described above, a number of patient- and tumor-specific data are collected. The following data are recorded after clinical information, macroscopic analysis of the tumor, histopathological and immunopathological analysis of tumor tissue and after analysis for driver mutations within the tumor cells respectively after analysis of miRNA in a miRNA-array approach (for 20 tumor cases and non-neoplastic lung parenchyma from the same patient) with more than 2500 miRNA spots for array hybridization. These data and image data from the primary tumor prior to surgery as well as data on Ki67-index and angiogenesis from the resected tumor specimen are provided in ObTiMA, similar to as described in the other cancer types (figure 3.4 and 4.4).

The following data are collected and provided:

- Patient data: pseudonym, gender, date of birth, vital status, date of decease, survival period
- Surgical data: specimen, frozen tissue availability, tumor size, primary or recurrent, surgery modality, surgery site
- Pathology data: variants of adenocarcinoma, tumor grade, stage grouping,
- Immunohistochemistry data: TTF-1, Ki-67, CD34, proliferation index, microvessel density
- Genetic data – mutations for: EGFR exon 18, 19, 20, 21, KRAS exon 2, exon 3, exon 4, EML4-ALK, BRAF exon 11, BRAF exon 15
- miRNA data
- Clinical data on chemotherapy: number of cycles, date of start and finish of each given cycle, specific drugs given for each cycle
- Diagnostic radiology and radiotherapy: CT, PET, PET/CT, number of sessions, date of start and finish of each session, radiation regimen

All these data were stored in specifically created CRFs in ObTiMA, the data management system for the lung adenocarcinoma/NSCLC scenario.

5.4 Ethical considerations

An ethical approval by the Ethical Committee of the ‘Ärztekammer des Saarlandes’ exists for the analysis of retrospective data in NSCLC tumor specimen and can be found in deliverable D4.1 (Initial analysis of the ethical and legal requirements for the sharing of data) of the CHIC project.

6 Conclusion

This deliverable deals with the scenarios and data from defined patients from the nephroblastoma SIOP-2001 trial, glioblastoma multiforme patients from the HGG-2010 trial and patients with non-small cell lung cancer. For each of these cancer types the clinical scenario, generated data, data storage in ObTiMA and ethical considerations are described as the basis of the hypermodels to be developed.

The scenarios are adopted from clinical practice so that hypermodels which answer the clinical question can be created for each of the cancer types, in order to guarantee the clinical relevance of the project. The different questions are:

- Nephroblastoma: Will a given nephroblastoma in a patient respond to pre-operative chemotherapy by tumour shrinkage, yes or no?
- Glioblastoma Multiforme: Is a patient with certain clinical and pathological characteristics at diagnosis and its specific immune profile predisposed to become a long-term survivor (or on the contrary is predicted to have early relapse) in the context of a combined postoperative radiochemoimmunotherapy? Will immunotherapy in the form of DC vaccination be helpful to reach this long-term survival and if so, should this vaccination be given early or late?
- Non-Small Cell Lung Cancer: Does a patient with certain clinical and pathological characteristics at the time of diagnosis and surgical resection of the tumor tissue show characteristic tumor parameters enabling to predict the likelihood of recurrence and/or metastasis of the tumor or will they qualify for clinical surveillance if a relapse is unlikely? Moreover, can personalized, tumor mutation-specific therapies be helpful to prolong the time to recurrence and long-term survival?

The described and provided scenarios and data are used for building meta- and multiscale hyper-models and repositories for the integrated hypermodel based oncosimulator. The nephroblastoma and lung cancer hypermodel based oncosimulator will primarily be based on mechanistic mathematical models whereas the glioblastoma hypermodel based oncosimulator is primarily based on machine learning techniques. In the latter case it is crucial to identify those parameters that play a major role in the outcome from the highly complex glioblastoma data from combined therapy with neurosurgery, radiotherapy, chemotherapy and immunotherapy.

For optimal understanding between the clinicians and modellers, the scenarios and data are explained from the point of view of the clinicians as data provider.

7 References

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Appendix 1 – Abbreviations and acronyms

ADC	Apparent Diffusion Coefficient
APC	Antigen Presenting Cell(s)
BBB	Blood-Brain-Barrier
BCM20	Brain Cancer Module: supplemental questionnaire with 20 to 26 questions, specifically for patients with brain cancer
CBA	Cytometric Bead Assay
CCSK	Clear Cell Sarcoma of the Kidney
CD	Cluster of Differentiation
CDP	Center for Data Protection
CHIC	Computational Horizons In Cancer
CMN	Congenital Mesoblastic Nephroma
COG	Children's Oncology Group in North America
CRF	Case Report Form
CT	Computed Tomography
DC(s)	Dendritic Cell(s)
DCi	Immature Dendritic Cells
DCm	Mature Dendritic Cells
DCm-HGG-L	Mature Dendritic Cells loaded with tumor Lysate from the High Grade Glioma
DICOM	Digital Imaging and Communications in Medicine
ELISA	Enzyme-Linked Immuno Sorbent Assay
EOR	Extent of Resection
EORTC *QLQ-C30*	European Organisation for Research and Treatment of Cancer Quality of Life Questionnaire with 30 questions for cancer patients

FACS	Fluorescence-Activated Cell Sorting
FMH	Fertgkeitenskala Münster-Heidelberg
GBM	Glioblastoma Multiforme
GCP	Good Clinical Practice
HGG	High Grade Glioma
HGG-L	Tumor Lysate from the High Grade Glioma
IASLC	International Association for the Study of Lung cancer
IHC	Immunohistochemistry
KPS	Karnofsky Performance Status
MDSC	Myeloid Derived Suppressor Cells
miRNA	Micro RNA
MMSE	Mini-Mental State Examination
MRI	Magnetic Resonance Imaging
MRTK	Malignant Rhabdoid Tumors of the Kidney
NK(T)-cells	Natural Killer (T) cells
NSCLC	Non-Small Cell Lung Cancer
ObTiMA	Ontology based Trial Management Application
OS	Overall Survival
PACS	Picture Archiving and Communication System
PBMC	Peripheral Blood Mononuclear Cells
PET	Positron Emission Tomography
PFS	Progression Free Survival
PFS6m	Progression Free Survival after 6 cycles of maintenance chemotherapy
QoL	Quality of Life

RANO criteria	Response Assessment in Neuro-Oncology criteria
RCC	Renal Cell Carcinoma
RCT	Randomised Clinical Trial
RPA	Recursive Partitioning Analysis
rrCBV	relative regional Cerebral Blood Volume
RTV	Residual Tumor Volume
SIOP	International Society of Paediatric Oncology
SIOP-RTSG	International Society of Paediatric Oncology Renal Tumour Study Group
TMZ	Temozolomide
TMZm	Maintenance chemotherapy with Temozolomide
TMZr	Concomitant chemotherapy during radiotherapy with Temozolomide
TNM	Classification of malignant tumors based on Tumor size, affected lymph Nodes and Metastasis
Tregs	Regulatory T cells
UICC	Union for International Cancer Control
USAAR	Universitaet des Saarlandes
UZ/KU Leuven	Universitair Ziekenhuis/Katholieke Universiteit Leuven (in Dutch) University Hospital/Catholic University Leuven
WBC	White blood cells
WHO	World Health Organization
WT	Wilms tumour (or nephroblastoma)