Center for Personalized Cancer Treatment (CPCT)

Development of a platform for next-generation DNA sequencing based personalized treatment for cancer patients: Protocol to obtain biopsies from patients with locally advanced or metastatic cancer (CPCT - 02 biopsy protocol)

ToetsingOnline nr: NL35781.041.11

Study coordinator

N. Steeghs, MD/PhD
University Medical Center Utrecht
Heidelberglaan 100
3584 CX Utrecht
088 - 755 55 55

History of amendments:

01-08-2011	**Protocol approval**
21-09-2011	**Amendment 1**
03-10-2011	**Approval Amendment 1**
06-03-2012	**Amendment 2**
22-03-2012	**Approval Amendment 2**
17-09-2012	**Amendment 3**
26-10-2012	**Approval Amendment 3**
13-08-2013	**Amendment 4**
30-09-2013	**Approval Amendment 4**
19-06-2014	**Amendment 5**
07-07-2014	**Approval Amendment 5**
29-07-2014	**Amendment 5.1**
31-07-2014	**Approval Amendment 5.1**
16-01-2015	**Amendment 5.2**
18-02-2015	**Approval Amendment 5.2**
06-03-2015	**Amendment 6.0**
07-04-2015	**Approval Amendment 6.0**
07-12-2015	**Amendment 7.0**
03-05-2016	**Approval Amendment 7.0**
23-11-2016	**Amendment 8.0**

Signature page

Study title:

Development of a platform for next-generation DNA sequencing based personalized treatment for cancer patients: Protocol to obtain biopsies from patients with locally advanced or metastatic cancer (CPCT - 02 biopsy protocol)

Study coordinator / sponsor signature

N. Steeghs, MD/PhD
University Medical Center Utrecht
Heidelberglaan 100
3584 CX Utrecht
The Netherlands
oncology-trials-secretariat@umcutrecht.nl

_____
Signature

_____
Date

Table of contents:

1 Protocol synopsis

Study title	Development of a platform for next-generation DNA sequencing based personalized treatment for cancer patients: Protocol to obtain biopsies from patients with locally advanced or metastatic cancer (CPCT - 02 biopsy protocol)
Rationale	A major challenge for researchers in cancer care is to expedite the development of new therapeutics and the Center for Personalized Cancer Treatment (a collaboration of the Dept. of Medical Oncology from the University Medical Center Utrecht, Netherlands Cancer Center - Antoni van Leeuwenhoek hospital and the Erasmus MC Cancer Institute and including other Dutch academic and affiliated hospitals) is an initiative to achieve this goal. The current and future generation anti-cancer drugs are developed to specifically activate or deactivate deregulated gene products or signaling pathways in cancer cells. The development of such “targeted” agents is an exciting new opportunity that promises to deliver more anti-cancer efficacy and less toxicity. Although targeted therapy has been a breakthrough in medical oncology leading to the development of a portfolio of potentially successful new drugs, it has not yet delivered the much needed relief for large patient populations. We believe that the development of these agents, as could also be postulated for several of the classical cytoxic agents, is mainly hampered by our lack of successful patient selection. The advent of NGS platforms enables us to probe a significant proportion of the cancer genome and thus to develop a realistic view on the complex genetic changes in cancer cells. The CPCT aims to use NGS platforms to improve the selection of patients for systemic therapy trials. We will obtain tumor biopsies of a (preferably) metastatic or locally advanced lesion and peripheral blood sample from all patients included in the trial; the biopsies to obtain information on the tumor related genetic mutations (mutational profile) and the blood samples to assess each patient’s germline DNA background variation. Review of the literature shows that in general tumor biopsies can be performed with only minor complications and acceptable risks. We will recruit patients with locally advanced or metastatic cancer for whom systemic treatment with anti-cancer agents is indicated and we aim to use the information obtained from DNA sequencing to investigate the predictive value of the mutational profile.

Study design	This is a multicenter study combining histological biopsy of tumor material with DNA sequencing using Next Generation Sequencing (NGS) platform. The study aims to obtain a more accurate pre-treatment stratification of cancer patients by obtaining fresh tumor biopsies for next-generation sequencing to obtain a mutational profile.
Objectives	<u>Primary objective:</u> To analyze the individual cancer genome in cancer patients to develop future predictors for response to systemic treatment <u>Secondary objectives:</u> • To determine the amount of biopsy samples with sufficient DNA for analysis • To determine the amount of biopsy samples with an adequate mutational profile • To collect and anonymously interpret all mutational profiles obtained using this protocol • To determine changes in the mutational profile under the influence of systemic treatment • To explore and analyze the individual microRNA,(phospho)proteomic profiles and organoid cultures in patients with cancer to develop future predictors for response to systemic treatment. • To explore the correlation between mutational profiles in solid tumor biopsies and liquid biopsies (circulating tumor DNA)
Endpoints	<u>Primary endpoint:</u> • Number of patients with adequate mutational profiles of their cancer genome and adequate follow up of systemic treatment efficacy. <u>Secondary endpoints:</u> • Percentage of samples with sufficient DNA for sequencing analysis • Percentage of samples with an adequate mutational profile that allows biomarker discovery efforts. These profiles will be deposited in the CPCT database. Database of all (anonymized) data obtained using this protocol • Differences in mutational profile pre, post and during treatment • Number of samples with an adequate microRNA, (phospo)proteomic profiles and organoid cultures that allows biomarker discovery efforts. These profiles will be deposited in the CPCT database. • Number of samples with a clear correlation between mutational profiles in solid and liquid biopsies
Patient selection	Main patient selection criteria, defined as inclusion criteria, are: • Locally advanced or metastatic cancer • Indication for new line of systemic treatment with anti-cancer agents • Measurable metastatic or locally advanced lesion(s), according to RECIST 1.1 criteria (or RANO criteria if applicable). For breast and prostate cancer patients with bone only disease, no measurable lesions need to be present.

	• Safe biopsy of a metastatic or locally advanced lesion possible • Age ≥ 18 yr • Expected adequacy to follow up • Written informed consent
Criteria for evaluation	DNA sequencing: DNA will be isolated from tumor biopsies of a metastatic or locally advanced lesion and blood, allowing us to identify tumor specific mutations, potentially guiding clinical decision-making. Obtained sequence data are processed using publicly available and custom made algorithms to identify genetic alterations. Additional filtering steps are applied to focus on those variants most likely affecting protein function, like non-synonymous-, splice-site- and nonsense mutations together with small insertions and deletions. We will also perform a signalling pathway analysis to identify pathways with aberrant activity, opening up potential intervention strategies.
Statistics	There are no formal statistical considerations that underlie the sequencing efforts. The primary endpoint is amount of patients that has adequate sequencing profiles and have adequate follow up data on the outcome of systemic treatment. We aim to determine the mutational profile of metastatic lesions in 500 patients a year. We will also report the percentages of samples with sufficient DNA for analysis and of samples with an adequate mutational profile.

2 Background and introduction

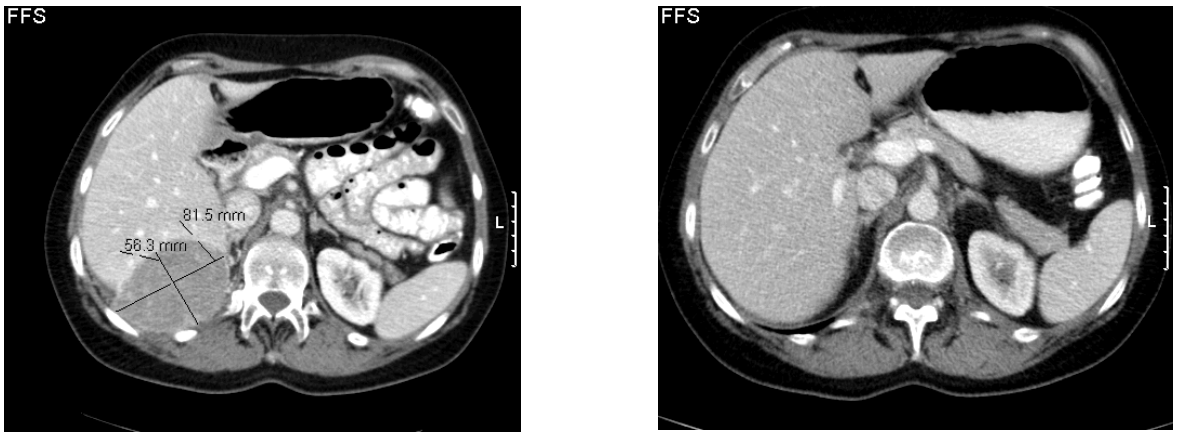
2.1 Center for Personalized Cancer Treatment

The development of new therapies for cancer patients has been slow despite a vast investment from both academia and industry.[1] A major challenge for researchers in cancer care is to expedite the development of new therapeutics and the Center for Personalized Cancer Treatment (CPCT, a collaboration of the Dept. of Medical Oncology from the University Medical Center Utrecht, Netherlands Cancer Center - Antoni van Leeuwenhoek hospital and the Erasmus MC Cancer Institute and including other Dutch academic and affiliated hospitals) is an initiative to help achieve this goal.

The mechanistic insight that basic research on cancer has generated is overwhelming and this wealth of data has resulted in a large pool of potential targets for anti-cancer therapy. The current and future generation anti-cancer drugs are developed to specifically activate or deactivate deregulated gene products or signalling pathways in cancer cells. The development of such “targeted” agents is an exciting new opportunity that promises to deliver more anti-cancer efficacy and less toxicity.

To illustrate the potential benefits of such an approach we show the CT scan (Fig.1) of a patient with a metastatic PEComa, a rare sarcoma variant. The patient had no further conventional treatment options. We found a TSC1 mutation that is known to activate the mTOR pathway and started treatment with the mTOR inhibitor everolimus. Within 3 months her complaints resolved and her tumor had gone into remission.

Figure 1



A patient with metastatic PEComa. Based on genetic alterations the mTOR pathway was specifically activated and the tumor had a high vascular density (left panel). This patient was treated with the mTOR inhibitor everolimus for 3 months, resulting in a complete remission (right panel).

Although targeted therapy has been a breakthrough in medical oncology leading to the development of a portfolio of potentially successful new drugs, it has not yet delivered the much needed relief for large patient populations. We believe that the development of these agents is mainly hampered by our lack of successful patient selection and that future treatment will no longer be based on the origin (organ) of the primary tumor, but on the mutational profile (predictive biomarkers) of the individual tumor. Several targeted agents have shown remarkable efficacy in patient populations selected based on biomarkers (table 1). The development of trastuzumab for the treatment of patients with HER2-overexpressing metastatic gastric or gastro-oesophageal (GE) junction adenocarcinoma is a good example. Recent

evidence suggests that trastuzumab is beneficial for patients with HER2+ gastric cancer[2]. While the fraction of HER2 expression between breast cancer and gastric cancer patients is similar (±22%), it took 12 years from registration of trastuzumab for the treatment of Her2+ breast cancer to its registration for Her2+ gastric cancer. We envision that this delay could be shortened if we include all tumor types in early phase trials.

However, for most of our targeted therapies, as could also be postulated for several of the classical cytoxic agents, we do not have these clean targets to aim for. Therefore, we need to develop new strategies and technologies to select those patients that respond to selected targeted agents. Selecting patients for targeted therapy based on molecular aberrations in the individual tumor better fits the development of targeted therapies as we target activated signalling pathways and not aspecific phenomena such as proliferation or metabolism.

Table 1: Examples of successful DNA based biomarkers and targeted therapies in oncology

Target	Disease	Drug	Success
Her2Neu	Breast, stomach	Herceptin, Lapatinib	HR: +/- 0.50 for progression free survival for Her2+ breast cancer patients after surgery[3;4]
c-KIT	GIST	Imatinib	Majority of patients show impressive responses[5]
BCR-Abl	CML	Imatinib	>50% response in BCR-ABL positive CML[6;7]
ALK	NSCLC	Specific ALK inhibitor	Promising phase I data, to be confirmed.
PARP	BRCA 1 / 2 associated ovarian carcinoma, triple negative breast cancer	Multiple PARP inhibitors	Promising phase I/II data[8;9]
BRAF	BRAF mutant melanoma	Specific BRAF inhibitor	Promising phase I data[10]

Current paradigm dictates that cancer is a genetic disease. In cancer cells the genome is mutated in such a way that the cancer cell acquires the seven hallmarks of cancer described by Hanahan and Weinberg [11], including self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis. The methodology to study the human cancer genome is rapidly improving and reading the sequence of (significant parts of) the genome is becoming relatively easy and affordable. Next Generation Sequencing (NGS) platforms, such as the SOLiD™ platform (Life Technologies), are transforming our ability to read and understand the alterations in the genome of cancer cells.[12] The use of such high-throughput technology for patient care could become cost-effective and is potentially superior in predicting therapy response compared to our standard diagnostic tests mainly focusing on specific mutations in a single gene or protein.

A major hurdle in generating accurate single feature predictive biomarkers is the tendency of signalling pathways to behave as parts of a self-containing system. These systems generally have redundancies and feedback mechanisms to overcome disturbances. Determining whether the targeted drug is inhibiting an essential component of the signalling cascade is virtually impossible without generating a more comprehensive insight into the overall genetic make-up of the cancer cell. The advent of NGS platforms enables us to probe a significant proportion of the cancer genome and thus to develop realistic view on

the complex genetic changes in cancer cells. The CPCT aims to use NGS platforms to improve the selection of patients for targeted therapy.

2.2 Patients

The CPCT aims to obtain NGS based mutational profiles combined with response data on systemic treatment. We will recruit patients with locally advanced or metastatic solid tumors with measurable disease before the start of systemic treatment. If possible we will use the information obtained from DNA sequencing to stratify patients for inclusion in clinical trials. Patients who will be recruited for the CPCT-02 biopsy protocol will be patients who are eligible for treatment with systemic anti-cancer agents, including but not limited to:
pazopanib/ sunitinib/ sorafenib/ axitinib/ regorafenib; everolimus; all aromatase inhibitors; exemestane combined with everolimus; tamoxifen; fulvestrant; vismodegib; panitumumab/ cetuximab; erlotinib/ gefitinib; vemurafenib/ other BRAF inhibitors and these in combination with MEKK inhibitors; ipilimumab; anti-PD1/anti-PD1L antibodies; imatinib; abiraterone/ enzalutamide; T-DM1.

2.3 Tumor biopsies

We will obtain a baseline tumor biopsy of a metastatic or locally advanced lesion and a peripheral blood sample from all patients included in this trial. These procedures are necessary to obtain reliable sequencing information; the biopsies to obtain information on the tumor related genetic mutations and the blood samples to assess each patient's germline DNA background variation. Determining patient's germline DNA background variation is essential for correct interpretation of the mutational profile. Differentiation between tumor relevant somatic mutations and irrelevant mutations (i.e. mutations that can be detected in healthy tissue/blood, like SNPs) is only possible by determining patient's germline DNA background variation. Only relevant somatic mutations will be used for determining the mutational profile which will be correlated to the response to therapy.

Patients will also be asked to undergo (optional) on-treatment and post-treatment biopsies. Post-treatment biopsies will take place at discontinuation of systemic treatment, either due to progressive disease, unacceptable toxicity of systemic treatment or any other reason. This allows us to determine direct effects of the systemic treatment on the mutational profile (in case of instant progression under treatment or instant unacceptable toxicity), long-term effects of the systemic therapy on the mutational profile (in case of discontinuation of treatment due to disease control) and to observe new mutations that cause insensitivity to systemic treatment (progressive disease after multiple cycles).

It is not possible to determine a fixed moment of the on-treatment biopsies. This differs not only on type of systemic treatment (based on mode of action and knowledge/expectation of time to response between chemotherapy, targeted therapy or immunotherapy), but mainly on type of response, for example extremes in response (e.g. great response when poor response is expected or the opposite) or patients with a mixed response (partial response of metastatic lesions combined with growing lesions or new lesions).
If tumor tissue becomes available during treatment because of other reasons, with the permission of the patient, this may be used for extra DNA analyses.

Standardized collection of adequate tissue samples is important in performing biomarker research. As a proof of concept, we have sequenced 1265 cancer related genes in 20 patients with metastatic colorectal

cancer in both the primary tumor and resected metastases. Preliminary analysis showed extensive variation in mutational profile between the primary tumor and its metastasis, with a shared mutation spectrum of only 51%. These differences include loss of mutations as well as gain of mutations. Two recent papers describe similar effects in breast cancer patients. The first paper compared the tumor genome from a patient with an ER positive primary breast cancer with a metastasis that occurred 9 years later. The metastasis contained 19/32 novel mutations compared to the primary tumor[13]. The second paper describes the tumor genome from a patient with basal like breast cancer arising 8 months after resection of her primary tumor. Here, the metastasis was significantly enriched for 20/40 mutations found[14]. These data have been validated by a similar sequencing approach in pancreatic cancer, once more showing that metastases are essentially derived from subclones of the primary tumor and may exhibit seeding organ specific genetic mutations[15;16]. Therefore, we need to probe the mutational profile of metastases to optimally guide therapy choice.

As patients will be asked to undergo an invasive procedure it is important to address the potential safety issues. Review of the literature shows that in general tumor biopsies can be performed with only minor complications and acceptable risks (Appendix B). Although biopsies are generally considered to be safe, the location of the tumor is an important determinant of the risk associated with the biopsy. The risk assessment of liver biopsies shows that overall mortality of ultrasound-guided large-core needle biopsy of liver tumors is very small, i.e. <0.5%. Large-core needle biopsies of liver lesions is performed, usually with a 18G biopsy needle and the use of a 17G guiding needle which is positioned into the target lesion. In general, 2-3 biopsy samples (specimen length 22mm) per lesion are obtained. Complications are generally limited when precautions are taken into account (e.g., adequate coagulation status). Bleeding risk in general is considered to be lower than 10% (Appendix B). In contrast, biopsies of lung metastases have a risk of causing pneumothorax, whereas biopsies of subcutaneous metastases are considered to be very safe. Overall, tumor biopsies provide important information and pose a minor burden to the patient's well being.

In a recent position paper, the Cancer and Leukemia Group B Ethics Committee defined 5 conditions to justify mandatory biopsies[17]:

1. There should be a strong scientific rationale for the study;
2. Meaningful efforts must be taken to ensure that patients are adequately informed that biopsies are required and are for research purposes only;
3. Adequate monitoring of safety of the procedure and reporting of adverse effects;
4. If possible samples should be obtained at the time of clinically required procedures to minimize discomfort and inconvenience;
5. Research biopsies should only be performed if samples cannot be obtained in a different way.

In this protocol we will try to adhere to these important conditions as far as they are relevant to our proposed efforts (point 1-4 will be specifically addressed).

3 Objectives and endpoints

3.1 Objectives

3.1.1 Primary objective

The primary objective of this study is:

- To analyze the individual cancer genome in cancer patients to develop future predictors for response to systemic treatment in individual patients with cancer.

3.1.2 Secondary objectives

Secondary objectives of this study are:

- To determine the amount of biopsy samples with sufficient DNA for analysis
- To determine the amount of biopsy samples with an adequate mutational profile
- To collect and anonymously interpret all mutational profiles obtained using this protocol
- To determine changes in the mutational profile under the influence of systemic treatment
- To explore and analyze the individual microRNA, (phospho)proteomic profiles and organoid cultures in patients with cancer to develop future predictors for response to systemic treatment
- To explore the correlation between mutational profiles in solid tumor biopsies and liquid biopsies (circulating tumor DNA)

3.2 End-points

3.2.1 Primary endpoint

The primary endpoint is:
Number of patients with adequate mutational profiles of their cancer genome and adequate follow up of systemic treatment efficacy.

3.2.2 Secondary endpoints

Secondary endpoints are:

- Percentage of samples with sufficient DNA for sequencing analysis
- Percentage of samples with an adequate mutational profile that allows biomarker discovery efforts. These profiles will be deposited in the CPCT database.
- Database of all (anonymized) data obtained using this protocol
- Differences in mutational profile pre, post and during treatment
- Number of samples with an adequate microRNA, (phospo)proteomic profiles and organoid cultures that allows biomarker discovery efforts. These profiles will be deposited in the CPCT database.
- Number of samples with a clear correlation between mutational profiles in solid and liquid biopsies

4 Trial design

This is a study combining histological biopsies of tumor material and blood sample with DNA sequencing using a Next Generation Sequencing (NGS) platform. The study aims to obtain a more accurate pre-treatment stratification of cancer patients by obtaining fresh tumor biopsies of a metastatic (preferably) or locally advanced lesion and a blood sample for next-generation sequencing to obtain a mutational profile.
All research analysis not mentioned in the objectives will be considered specific research questions for which separate approval is required, as outlined in chapter 17.

The study is a Dutch multicenter study and will be executed in the University Medical Center of Utrecht, the Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital (NKI-AvL) in Amsterdam and the Erasmus MC Cancer Institute in Rotterdam and will include other Dutch academic and affiliated hospitals.

5 Patient selection

Selection criteria, defined as inclusion criteria, are:

1. Patients with locally advanced or metastatic cancer for whom a new line of systemic treatment with anti-cancer agents is indicated. Both standard of care treatment or treatment with these compounds within clinical trials are included. A CPCT-02 biopsy may be combined with a diagnostic biopsy.
2. Measurable metastatic or locally advanced lesion(s), according to RECIST 1.1 criteria[18] (or RANO criteria if applicable). For breast and prostate cancer patients with bone only disease, no measurable lesions need to be present. Patients need to have evaluable disease, bone metastases that can only be detected on bone scans without CT/MRI or serum tumor marker will be considered inevaluable. Guidelines for response evaluation are given in appendix A and C.
3. Metastatic or locally advanced lesion(s) of which a histological biopsy can safely be obtained.
4. Patients age $\geq$ 18 years, willing and able to comply with the protocol as judged by the investigator with a signed informed consent.

Patients must meet selection criteria 3 not only prior to baseline biopsy, but also prior to the (optional) on-treatment and/or post-treatment biopsies.

6 Investigations and follow-up

6.1 Baseline screening

All patients should provide written informed consent before any study specific procedures are performed. Required baseline screening investigations after study enrollment (i.e. after written informed consent) are to be performed within 28 days before biopsy. Evaluations that have been performed within this period as part of standard of care will not have to be repeated. Baseline screening investigations include:

- Written informed consent
- Eligibility Screening Form
- History:
    - Medical history (with special interest in previous malignancies and previous chemotherapy) and demographics
- Pre-biopsy laboratory and physical examinations as per local guidelines.
- Radiological tumor assessment (contrast-enhanced CT or MRI) should only be performed during baseline screening if one of the following has not been established yet on a previous (recent) radiological evaluation:
    - Measurable metastatic or locally advanced disease (according to RECIST 1.1), with the exception of bone-only disease in breast and prostate cancer and response evaluation for patients with glioma (RANO criteria). For guidelines regarding response evaluation for patients with glioma (RANO criteria) refer to appendix A and for evaluation of bone-only disease refer to appendix C.
    - Safely accessible metastatic or locally advanced lesion(s) for biopsy
    - Baseline imaging studies should be done within 6 weeks before starting systemic treatment to allow for proper response evaluation.

6.2 Study related procedures

Study related procedures include:

- Histological biopsy of a (preferably) metastatic or locally advanced lesion (see CPCT-02 Lab Manual) for DNA sequencing: obligatory at baseline (pre-treatment), optional during and after treatment (on-treatment and post-treatment biopsies). Patients will be monitored in the hospital after biopsy according to local clinical protocol.
- Additional blood sample (see CPCT-02 Lab Manual) for determining patient's germline DNA background variation: obligatory at baseline only.
- Optional blood sample for liquid biopsy (circulating tumor DNA) at baseline, during treatment and at disease progression (see Chapter 7.4).
- Optional tissue biopsy of healthy tissue in selected patient groups (see CPCT-02 study manual) for correlation analyses between healthy and tumor tissue.

6.3 Follow-up

For all patients the treatment regimen(s) after biopsy will be recorded. The follow up for treatment regimen(s) will be recorded until: 1) treatment switch without additional biopsy before new treatment; 2) the decision to stop all systemic anti-cancer treatment or 3) death.

In general, for patients that undergo standard of care systemic anti-cancer treatment first treatment evaluation is after 8-12 weeks and every 12 weeks thereafter. The exact timing and method for disease assessment is depicted in the CPCT-02 Study Manual. Interval scans that are mandated by clinical decision making are allowed and shall be documented in the CRF.

After 1 year of systemic treatment evaluation intervals will be determined by the investigator.

If a patient undergoes another treatment, the patient can be asked again for further participation in the trial. With permission of the patient, more biopsies will be performed during the next treatment.

6.4 Off trial

Patients are considered off trial either 14 days after definitive discontinuation of the initiated systemic treatment (if patients refuse additional biopsy) or 48 hours after the final histological biopsy.
Note that patients may undergo more than one treatment.

6.5 Evaluability of patients

Whether or not patients are evaluable for the primary and secondary endpoints differs between endpoints. For each endpoint evaluability criteria are established.

6.5.1 Primary endpoint

All patients who underwent the study related histological biopsy and blood sampling for DNA sequencing will be evaluable for the primary endpoint (i.e. Number of patients with adequate mutational profiles of their cancer genome and adequate follow up of systemic treatment efficacy.).

6.5.2 Secondary endpoints

6.5.2.1 Sufficient DNA for sequencing analysis

Patients are evaluable for this secondary endpoint if study related histological biopsy and blood sampling for DNA sequencing was performed. Patients are non-evaluable though when study related biopsy samples had to be sacrificed for (prioritized) diagnostic reasons (see also Chapter 7.3 Storage of samples).

6.5.2.2 Adequate mutational profile that allows biomarker discovery.

Patients are evaluable for this secondary endpoint if study related histological biopsy and blood sampling for DNA sequencing was performed. Patients are non-evaluable though when study related biopsy samples had to be sacrificed for (prioritized) diagnostic reasons (see also Chapter 7.3 Storage of samples).

6.5.2.3 Database of all (anonymized) data

All patients who underwent the study related histological biopsy and blood sampling for DNA sequencing will be evaluable for the primary endpoint (i.e. the percentage of patients enrolled in clinical intervention trials based on the mutational profile of their cancer genome).

6.5.2.4 Differences in mutational profile between biopsies

Patients are only evaluable for this secondary endpoint when on-treatment and/or post-treatment biopsies have taken place, as well as baseline histological biopsy and blood sampling. Patients are non-evaluable though when baseline biopsy samples had to be sacrificed for (prioritized) diagnostic reasons (see also Chapter 7.3 Storage of samples).

6.6 Summary table

Study assessments are summarized in table 2.

Table 4: Study assessments

	Baseline				Treatment	Additional biopsies (optional)					
	Baseline screening	Study related procedures			Recording clinical data	On-treatment biopsy			Post-treatment biopsy		
Day	- 28 to -1	1				Y			Z		
Written informed consent [a]	X										
Eligibility Screening Form	X										
History [b]	X										
CT/MRI [c]	X				X[j]						
Laboratory examinations [d]	X	X			X[k]	X			X		
Blood sample for germ-line DNA analysis [e]		X									
Histological biopsy metastatic lesion [f]		X				X			X		
Optional histological biopsy of healthy tissue [h]		X				X			X		
Optional blood sample for liquid biopsy (ctDNA)[l]		X				X			X		
Follow up after biopsy		X[g]				X[g]			X[g]		

[a] Written informed consent must be obtained before any study specific procedures, including baseline screening assessments; [b]Including contraindications for study related procedures; [c]Not obligatory when measurable metastatic or locally advanced disease (according to RECIST 1.1) and safety of histological biopsy can be established on recent previous radiological assessments, however baseline tumor measurements should not be older than 6 weeks before start of systemic treatment; [d] pre-biopsy laboratory examinations as directed per local institutional guidelines [e]1 x 10 ml CellSave blood (refer to lab manual); [f] Only after establishing no contraindications for biopsy; [g]Patients will be monitored in the hospital according to local protocol; [h]only in selected patient groups (as described in the CPCT-02 study manual) [j]schedule of imaging assessment according to the CPCT-02 Study Manual , baseline evaluations should ideally be available up to 6 weeks before start of treatment; [k]Tumor assessments according to appendix C should preferably be performed set intervals (as described in the CPCT-02 study manual) for breast and prostate cancer patients with bone only disease that is not measurable according to RECIST; [l] Optional blood samples for liquid biopsy (ctDNA) are implemented for patients with certain treatments and tumor types (see CPCT-02 Study Manual)

7 Study related procedures

7.1 Blood samples for DNA sequencing

A baseline blood sample (see CPCT-02 Lab Manual) for DNA sequencing will be taken preferably combined with baseline laboratory examinations. The blood sample will be used to determine the patient's germline DNA background variation.

7.2 Biopsy

An assessment will be made on the availability of a safely accessible metastatic lesion. Only patients with a safely accessible lesion will be included. Histological biopsy will be performed according to local institutional guidelines. Registration is needed on which metastatic or locally advanced lesion the biopsy has been performed. In a selected patient group an optional tissue biopsy of healthy tissue will be performed for correlation analyses between healthy and tumor tissue (see CPCT-02 study manual). This is optional. At all time points (preferably) of tumor biopsy (pre-treatment, on-treatment, at tumor progression) the tumor biopsy can be combined with one optional biopsy of healthy tissue in this selected patient group (see CPCT-02 study manual).

7.3 Storage and use of samples

Blood and tissue samples will be stored in the biobank at the Department of Pathology at the Netherlands Cancer Institute – Antoni van Leeuwenhoek. All samples will be anonymized by using a unique patient identification code (see also Chapter 13.8 Confidentiality of patients). Data can be shared and combined with data from other research institutes or commercial (pharma) partners. The CPCT will ensure that all data will be anonymized by using an unique patient identification code, which these external research partners can not trace back to the individual patient. Material of all biopsies will be used for DNA isolation and sequencing. Any remaining tissue will be used for internal validation of the sequencing method and for future, directly related research. The directly related research includes, but is not limited to: proteomics, RNA sequencing and culturing cells to obtain organoid cultures.

If the study related biopsy procedure was combined with histological biopsy for diagnostic reasons, the CPCT biopsy samples will remain stored until diagnostic pathological assessment has been completed. When diagnosis cannot be assessed on the diagnostic biopsy samples, the CPCT biopsy samples will be pulled out of the study and will be used for diagnostics (which we prioritize for the patient's wellbeing). In the unlikely event that even diagnostic assessment cannot be completed on the CPCT biopsy samples, patients can be asked for renewed diagnostic biopsy combined with study related histological biopsy.

7.4 Liquid biopsy (circulating tumor DNA)

An optional blood sample for sequencing of circulating tumor DNA (ctDNA) will be taken at baseline, every 8-12 weeks during treatment and at disease progression. In selected patients, samples will also be taken at additional time points when relevant, for instance at tumormarker nadir, maximum tumor regression, or in the first months of treatment (see CPCT-02 Study Manual). Optional blood samples have a maximum of 1 sample per week (in first months of treatment) and a maximum total of 15 samples per year). Samples are preferably combined with baseline or routine laboratory examinations. The blood sample will for instance be used to determine the patient's mutational profile in circulating tumor DNA. Not for all anti cancer treatments or tumor types ctDNA will be in place (also depending on finances). Patients for which ctDNA evaluation is possible are described in the CPCT-02 Study Manual. Depending on specific anti cancer treatments and tumor types, samples are send to the appropriate CPCT laboratories (for instance at the University Medical Center Utrecht, Netherlands Cancer Center - Antoni van Leeuwenhoek hospital or the Erasmus MC Cancer Institute) as described in the CPCT-02 Study Manual.

8 Next generation sequencing (NGS)

NGS platforms allow us to resequence large regions of the human genome in a fraction of the time and costs compared to conventional Sanger sequencing approaches. The main limitation of Sanger-based sequencing protocols is the separate PCR-based amplification of each interesting region and sequencing of individual samples. Next-generation sequencing platforms are based on a single reaction amplification of single molecules and subsequent massive parallel sequencing of millions of molecules. Additionally, we are able to isolate specific regions of interest from genomic DNA samples before the sequencing reaction, thereby excluding the analysis of irrelevant genes or repetitive regions.

Obtained sequence data are processed using publicly available and custom made algorithms to identify genetic alterations. Additional filtering steps are applied to focus on those variants most likely affecting protein function, like non-synonymous-, splice-site- and nonsense mutations together with small insertions and deletions. We will also perform a signalling pathway analysis to identify those pathways with aberrant activity, opening up potential intervention strategies.

9 Statistical considerations

There are no formal statistical considerations that underlie these sequencing efforts. Our primary endpoint is the number of patients with adequate mutational profiles of their cancer genome and adequate follow up of systemic treatment efficacy. We aim to determine the mutational profile of metastatic lesions in 500 patients a year. Since it is not possible to estimate the percentages of the various components of the process (depicted in Figure 2), we will report the percentage samples with sufficient DNA for analysis and the percentage of samples with an adequate mutational profile within three months after the first twohundred patients were subjected to a biopsy in any of the CPCT studies. We will also report the percentages of the various components of the process on a yearly basis to the METC.

10 Safety

The risk of blood draws (laboratory examinations at baseline screening, as well as additional blood sample for DNA sequencing) is negligible. The risk of biopsies is considered low based on a literature review on the safety of tumor biopsies, depicted in Appendix B. All safety measures and procedures will be performed according to local guidelines. No (severe) adverse events are scored within this protocol.

11 Forms and procedures for collecting data

11.1 Case Report Form (CRF)

Data for this study will be captured via a, study specific, Electronic Data Capture (EDC) system / electronic Case Report Form (eCRF).

11.2 Data flow

The CRF must be completed by the investigator or assigned/delegated Local Data Manager as soon as the requested information is available. The goal will be to complete the relevant sections of the CRF within one month after the data are available. The list of staff members authorized to sign case report forms (with a sample of their signature) must be sent to the Central Data Center by the responsible investigator before the start of the study.

In all cases the investigator remains responsible for the content of the completed CRFs.

The monitor will perform source document verification as described in the monitoring plan (Chapter 12: Data Monitoring).

The Central Data Center will check and process the answers from the investigator or Local Data Manger on automatically generated queries (those queries are generated “real time” along with data entry by the investigator or Local Data manager) from the eCRFs on a regular basis. Other irregularities found during this process will be queried as well. Those queries, attached to the corresponding eCRF page, must be answered by the investigator or Lokal Data manager within a reasonable time frame (the goal is within 1 month).

When a CRF of a specific patient is complete and contains no inconsistencies or irregularities the CRF will be considered “clean” and will be locked for data entry.

12 Data monitoring

12.1 Risk classification and monitoring intensity

Based on a literature review on the safety of tumor biopsies, the risk of biopsies is considered low with a small risk on significant/major complications (0 to 1.6%) or death (0 to 0.48%). The results of this literature review are depicted in Appendix B. The risk of blood draws for pharmacogenetic analysis is considered negligible.

Considering the guideline by the NFU (Dutch Federation of University Medical Centers) about quality insurance in human research ("Kwaliteitsborging van mensgebonden onderzoek") and the adapted guideline by the UMC Utrecht (based on the NFU guideline), we qualify the risk of this study as 'negligible' (small chance of moderate damage). According to this negligible risk a 'minimally intensive monitoring' is advised, which will be performed by a clinical research associate (CRA; monitor).

12.2 Data verification, frequency of monitoring and reporting

Data verification, the frequency of monitoring and the reporting of the monitor will be performed as described in the monitoring plan.

13 Ethics

13.1 Local regulations/Declaration of Helsinki

The investigator will ensure that this study is conducted to the standards of Good Clinical Practice, in full conformance with the “Declaration of Helsinki” (latest amendment), the Dutch laws and regulations and with the W.M.O. (“Wet Medisch-wetenschappelijk Onderzoek met mensen”) in particular.

13.2 Informed consent

Before patients agree to participation in this trial, they will be provided with written information (Patient Information Sheet). It is the responsibility of the investigator, or a person designated by the investigator, to obtain written informed consent from each subject participating in this study, after adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. Once the essential information has been provided to the patient and the investigator is satisfied with the candidate understanding of the implication of participating in this study, the subject will be asked to give consent by signing and dating the informed consent form in the presence of the (sub)investigator. The informed consent must be obtained before initiation of any study specific procedures, including specific screening procedures. Patients have the right to withdraw from the study at any time, without giving an explanation and without prejudice to their subsequent care.

The Informed Consent Form as well as the Patient Information Sheet must be prospectively approved by the Independent Ethics Committee/Institutional Review Board (IRB/IEC) and must be in compliance with Good Clinical Practice, local regulatory requirements and legal requirements. The investigator will retain the original of each subject’s signed Informed Consent Form.

13.3 Risk and benefit statement

Patients have a small chance of serious damage (as described in Chapter 10.1 and Appendix B), which requires patient commitment. It is our goal to enroll as many patients as possible into trials specifically designed to match the mutational profile found. As this methodology is experimental, we are currently unsure about the potential benefit patients can derive from this selection and we clearly emphasize the experimental nature of this protocol. We believe though that the possible benefits for the individual subjects as well as the general population are important as patient selection in oncology is a major issue. The only way to obtain possible predictive biomarkers (mutational profiles predicting response to chemotherapy) using DNA sequencing is to obtain material using a biopsy of the tumor itself. In our opinion the possible benefits outweigh the potential risk for the individual patients.

13.4 Patient communication

We will clearly state in the patient information sheet that it is our goal to enrol as many patients as possible to match the mutational profile found to efficacy of treatment. As this methodology is experimental we are currently unsure about the potential benefit patients can derive from this selection and we will clearly emphasize the experimental nature of this protocol. Patients will be advised on the optimal treatment we feel can be derived from the results of their individual mutational profile if possible. Advancing insights on the interpretation of the mutational profile and possible consequences for the therapy choice may also be discussed with patients.

13.5 Independent Ethics Committee/Institutional Review Board

This protocol and any accompanying material provided to the subject (such as the Patient Information Sheet and Informed Consent Form) will be submitted by the investigator to an Independent Ethics Committee. Approval from the committee must be obtained before starting the study and should be documented in a letter to the investigator specifying the date on which the committee met and granted the approval. Any modifications made to the protocol (amendments) after receipt of the Independent Ethics Committee approval must also be submitted by the investigator to the Independent Ethics Committee in accordance with local procedures and regulatory requirements. The only circumstance in which an amendment may be initiated prior to IRB/IEC approval is when the change is necessary to eliminate apparent immediate hazards to the subjects. In this case the accrual of new patients will be halted until IRB/IEC approval has been obtained.

13.6 Insurance

All participating sites will have a liability insurance which is in accordance with article 7, subsection 6 of the WMO.
The participating sites also have an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study:

1. € 450.000,- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the research;

2. € 3.500.000,- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the research;

3. € 5.000.000,- (i.e. five million Euro) for the total damage incurred by the organization for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study

13.7 Independent physician

In accordance with Dutch law and the W.M.O. for each center an independent physician who is not otherwise involved in this study and has agreed to act as the independent physician. The independent physicians will be available. Patients invited for participation will be offered the opportunity to gain further independent information on potential study participation.

13.8 Confidentiality of patients

All records identifying the patients will be kept confidential and will not be made publicly available. Strict measurements will be undertaken to protect confidentiality of DNA sequencing data. Study results and patient material (DNA sequencing results) will be processed using a unique patient identification code. Two separate databases will be installed; one database will contain the mutational profile of the tumor based on DNA sequencing data, as well as the unique patient identification code. The second database will contain the information reported in the CRF's, as well as the unique patient identification code.

The principal investigators should keep a patient enrolment log showing personal identifying information. Principal investigators and authorized personnel (depicted in the authorization log) will have access to medical records. Access to medical records for verification and auditing purposes by the accredited METC, regulatory authorities (e.g. inspectors of the Dutch Health Care Inspectorate), auditors and monitors will be required and permission from each subject will be obtained as part of the consent process.

14 Definition end of trial

End of trial is defined as the time at which all patients are considered off trial, i.e. either 14 days after definitive discontinuation of the initiated systemic treatment (if patients refuse post-treatment biopsy) or 48 hours after the final histological biopsy.

15 Sponsorship statement

This is an investigator-initiated trial and therefore the study coordinator is the investigator-sponsor. To ensure compliance with the GCP-ICH guidelines the investigator-sponsor will contract a qualified person to monitor the study.

16 Publication of trial results

Publication of trial results must be reviewed by the study steering committee of the CPCT (Center for Personalised Cancer Treatment) after completion of the trial or at any moment this committee deems publication advisable. All members of the writing committee will also be offered the opportunity of reviewing any publication. The order in which authors will be mentioned in publications will be determined by the study steering committee and the principal investigators.

We foresee that the data acquired from this protocol will result in multiple publications. As described in the letter of intent signed at the foundation of the CPCT all sites will equally benefit from these data. Furthermore this protocol and the data that are captured will be used to support a database for systems biology enabling research.

17 Use of CPCT-materials for specific research questions

When material collected as part of this protocol CPCT-02 is to be used to investigate specific research questions beyond the scope of this protocol, prior approval will be obtained according to the NKI-AVL Biobank Regulations ("NKI-AVL Biobank Reglement"). Researchers together with the responsible CPCT researcher will submit a research proposal form to the Non Executive Board of CPCT. With the approved proposal form the researcher will be informed that the sample and data request can be submitted to the Institutional Review Board (IRB) by the procedure as described in the '"NKI-AVL Biobank Reglement" and approved by the Biobank Committee and Board of Directors of the NKI-AVL.

During the review, the Institutional Review Board (IRB) will take the scientific value of the proposal into account and will judge if the proposal is in accordance with the rights and Broad Consent of the donors that originally donated their material to the CPCT-Biobank.

18 Appendices

18.1 Appendix A: Tumor Evaluation Criteria

18.1.1 RECIST-criteria, version 1.1

A.1. Measurability of tumor at baseline

At baseline tumor lesions/lymph nodes will be categorized measurable or non-measurable as follows:

A.1.1. Measurable lesions

All measurements should be recorded in metric notation, using callipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start and never more than 4 weeks before the beginning of the treatment.

Tumor lesions:

Measurable tumor lesions are defined as those that can be accurately measured in at least one dimension (longest diameter in the plane of measurements is to be recorded) with a minimum size of:

10 mm by CT scan (CT scan slice thickness no greater than 5 mm);

10 mm caliper measurement by clinical exam (lesions which can not be accurately measured with calipers should be recorded as non-measurable;

20 mm by chest X-ray

Lymph nodes:

To be considered pathologically enlarged and measurable, a lymph node must be $\geq$ 15 mm in short axis when assessed by CT scan (slice thickness recommended no greater than 5 mm). At baseline and follow-up only the short axis will be measured and followed.

A.1.2. Non-measurable lesions

All other lesions, including small lesions (longest diameter < 10 mm or pathological lymph nodes with $\geq$ 10 to < 15 mm short axis) as well as truly non-measurable lesions. Lesions truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory

breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical examination that is not measurable by reproducible imaging techniques.

A.1.3. Method of assessment

The same method of assessment and the same technique should be used to characterise each lesion at baseline and during follow-up. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions:

Clinical lesions will only be considered measurable when they are superficial and P10mm diameter as assessed using callipers (e.g. skin nodules). For the case of skin lesions, documentation by colour photography including a ruler to estimate the size of the lesion is suggested. As noted above, when lesions can be evaluated by both clinical exam and imaging, imaging evaluation should be undertaken since it is more objective and may also be reviewed at the end of the study.

CT/MRI:

CT is the best currently available and reproducible method to measure lesions selected for response assessment. Measurability of lesions on CT scan is based on the assumption that CT slice thickness is 5mm or less. When CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans). If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

A.2. Tumor evaluation

A.2.1. Assessment of overall tumor burden and measurable disease

Overall tumor burden at baseline needs to be estimated to use as comparator for subsequent measurements to assess objective response or future progression. Only patients with measurable disease at baseline should be included in this protocol.

A.2.2. Baseline documentation of 'target' and 'non-target' lesions

All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total representative of all involved organs should be identified as *target lesions* and be recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs and their suitability for accurate reproducible repeated measurements.

A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the *baseline sum diameters*. The baseline sum

diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

All other lesions (or sites of disease) should be identified as *non-target* lesions and should also be recorded at baseline. Measurements for non-target lesions are not required and these lesions should be followed as 'present' or 'absent'.

18.1.2 Response evaluation for patients with glioma (RANO criteria)

General method of response assessment

Response to treatment is assessed on the basis of a set of target lesion(s) chosen before the first treatment administration (the complete list of target lesions must be reported on the initial measurement form before the start of treatment). These lesions must initially be measured in their two perpendicular dimensions, and these measurements must be repeated at each evaluation of the disease by the same method. Response evaluation is based on neuro-radiological imaging (MRI). For this protocol objective response (complete, partial response) and progression will be assessed by MRI. Objective response will only be assessed in patients not having undergone second surgery with complete removal of contrast enhancing lesions prior to study entry. For these patients measurable disease is required, which is defined as a clearly enhancing tumor with at two perpendicular diameters at entry equal or superior to 1 cm.
The contrast enhancing area will be considered as the basis for the tumor size assessment. Tumor size is defined as the product of the two largest perpendicular diameters. Only reductions in cross-sectional areas of 50% or more when calculating the response, the baseline scan must be used for initial comparison. In initially responding (≥ 50% reductions in cross-sectional areas) or stabilized (<50% reduction and < 25% increase in cross-sectional areas) patients, new scans must be compared to the nadir, this is the scan showing the maximum response (= minimum tumor size) during/after treatment. In assessing response, changes on T2 weighted images must be taken into consideration. For this protocol we are aiming at measuring quantitatively the FLAIR/T2 changes as well.

Definition of target lesions

Only the following lesions are eligible as target lesions:
• MRI contrast enhancing lesions with two perpendicular diameters of 10 mm or more visible on 2 or more axial slices which are 5 mm apart.
• Target lesion(s) must be measurable in two perpendicular diameters. In most patients, only one lesion will be present. In case of multifocal disease, a minimum of 2 lesions and maximum of 5 largest enlarging lesions will be chosen as target and the sum of the products of the perpendicular diameters will be determined.
All other lesions than target lesions, if applicable, are assessed according to the same schedule. They are only taken into account in two situations:
• If one of them clearly progresses, the overall response to therapy will be evaluated as "progression", independent of the response of target lesions
• All lesions must have completely disappeared to report a "complete response".

Adequate investigations must be carried out at each evaluation of the disease to detect eventual new lesions. If any new lesion is found, the response will be evaluated as "progression".
Regardless of the status of enhancing lesions, if progressive lesions are observed on T2 weighted images or FLAIR images, the patient will be considered radiologically progressive, but treatment may continue if this is considered to be in the best interest of the patient and there are no signs or symptoms of clinical progression.
By definition, non-target lesions are those that do not meet the criteria for target lesion.

Evaluation of patient treated after re-operation

Postoperative changes on contrast enhanced neuro-imaging may interfere with disease evaluation. Within the first three days after surgery on MR imaging a thin linear enhancement may develop around the resection cavity, thereafter this enhancement may become thick and nodular. Enhancement of dura and meninges may be more pronounced, even within the first days. The postoperative linear enhancement may persist up to 3-6 months, dural and meningeal enhancement may last much longer. If MRI made within 48 hours after surgery shows enhancing lesions with a nodular or mass like appearance in areas showing tumor on the pre-operative scans this is highly suggestive of residual tumor. The use of diffusion weighted MR imaging in the immediate postoperative MRI may help with the identification of
ischemic areas around the surgical cavity that may show enhancement with further follow-up.

Schedule of disease evaluation

The initial assessment of disease (including measurement of all target lesions) must be performed in the 4 weeks preceding start of study treatment.

Response Assessment in Neuro-oncology (RANO) criteria [Wen, P.Y., et al., *Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group.* J Clin Oncol, 2010. **28**(11): p.1963-72.**]**

RANO criteria were developed in response to the advances in imaging technology and current treatment practices in the era of anti-angiogenesis. The RANO criteria also place emphasis on the non-enhancing part of the tumor as shown on T2SE-weighted and FLAIR image sequences in contrast to the classical Macdonald criteria based on changes in area of enhancing lesions which is non-specific: the contrast area primarily reflects the passage of contrast material across the disrupted blood-tumor barrier. The standardized EORTC Brain Tumor Imaging Protocol may be used [Ellingson, B.M., et al., *Consensus recommendations for a standardized Brain TumorImaging Protocol in clinical trials.* Neuro Oncol, 2015. **17**(9): p. 1188-98].

Definition of response

For this trial, the primary measure of response and progression will be determined by the locally assessed response according to the modified RANO criteria. All treatment decisions should be based on the modified RANO criteria. Follow up assessments will be done using both Macdonald (T1 plus contrast) and modified RANO (while considering T2/FLAIR). Response and progression will be assessed by both sets of criteria.
Target lesions are measured in their two largest perpendicular diameters. Their area is conventionally calculated as the product of these diameters. In case of multifocal disease with

more than one target lesion, the total tumor size is calculated as the sum of the area of all target lesions.
Response is defined as follows according to the modified RANO criteria, which also consider T2 weighted and FLAIR images:
Complete response (CR): Requires all of the following: 1) Complete disappearance of all enhancing measurable and non-measurable disease; 2) No new lesions; 3) Stable or improved non-enhancing abnormalities on FLAIR/T2 images as compared to baseline 4) Patients must be off corticosteroids (or on physiologic replacement doses only) and stable or improved clinically.
Partial response (PR): Requires all of the following: 1) Only reductions of cross sectional areas of 50% or more will be considered a response; when calculating the response, the baseline MRI must be used for comparison ; 2) No progression of non-measurable disease; 3) No new lesions; 4) Stable or improved non-enhancing abnormalities on FLAIR/T2 images as compared to baseline 5) Patients should be on
a corticosteroid dose that should not be greater than the dose at time of baseline scan, and should be stable or improved clinically.
Progressive disease (PD): Is defined by any of the following: 1) ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions (over baseline if no decrease) on stable or increasing doses of corticosteroids; 2) ≥ 25% increase in sum of the products of perpendicular diameters of area's with abnormalities on FLAIR/T2 images compared to the nadir time point (point with the smallest FLAIR/T2 abnormalities, even if still improved as compared to baseline, and on stable or increasing dose of steroids 3) The appearance of any new lesions; 4) Clear progression of non-measurable lesions; 5) Definite clinical deterioration not attributable to other causes apart from the tumor, or decrease in corticosteroid dose.
If the evidence of PD is equivocal (target or non-target), treatment may continue until the next assessment, but if PD is confirmed at the next follow up, the earlier date must be used as the date of progression
Stable Disease: This occurs if the patients did not qualify for CR, PR, or PD (see below) and requires: 1) No meaningful change in the appearance of the FLAIR/T2 images compared to baseline or to the nadir (point with the smallest FLAIR/T2 abnormalities) if a decrease occurred. 2) The patient should be stable clinically. In the event the steroid dosage has been increased for new signs and symptoms without confirmation of disease progression on imaging, and further follow-up imaging shows that with hindsight this increase in steroids was indeed unequivocally needed due to disease progression, the date of progression will be the date steroids were increased.
For patients operated at recurrence and without measurable or non-measurable disease post-surgery, any new appearance of tumor will qualify for PD. In case non measurable tumor is left after surgery i.e. tumor less than 10 mm, unequivocal progression of non-target disease may be accepted as evidence of disease progression, where the overall tumor burden has increased sufficiently to merit discontinuation of treatment. Modest increase in the size of a non-target lesion is NOT considered unequivocal progression. If the evidence of PD is equivocal (target or non-target), treatment may continue until the next assessment, but if confirmed, the earlier date must be used as the date of progression. This implies that in case of gross total resection of the enhancing lesion, if at follow up minimal enhancement of unclear significance arises, treatment may continue until further follow-up gives unequivocal evidence of tumor follow-up.
To ensure a homogeneous radiological evaluation, all MRI images will be centrally reviewed as a secondary analysis and specific study MRI protocol will be used.

	CR	PR	SD	PD
T1 gadolinium enhancing disease	None	≥ 50% ↓	≥ 50% ↓ but <25% ↑	≥ 25% ↑
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	≥ 25% ↑
New lesion	None	none	none	present
Coricosteroids	None	Stable or ↓	Stable or ↓	Not applicable*
Clinical status	Stable or ↑	Stable or ↑	Stable or ↑	↓
Requiements for response	All	all	all	Any

*increase in steroids alone does not qualify for PD

Overall response

The overall response is evaluated at each assessment of the disease. If PD exists in any lesion, or when a new lesion appears, then the overall result will be PD. Progression in non-measurable lesions leading to define deterioration of the patient due to tumor bulk should be taken to indicate progression, regardless of what happens in measurable disease.

• Best overall response: best overall response is the best response designation recorded from the date of study inclusion until disease progression.

• Objective response: objective response includes best overall responses CR and PR.

18.2 Appendix B: Safety of tumor biopsies

B.1 Risk of tumor biopsies

The results of a literature review on the safety of tumor biopsies are depicted in this table.

Safety of tumor biopsies									
Author	**Study design**	**No. of patients (pts)**	**No. of biopsies (bps)**	**Overall mortality**	**Mortality in patients with malignancy**	**Significant or major complications**	**Pain**	**Bleeding**	**Complications associated with malignancy**
Myers, 2008[19]	Retrospective review of records (Percutaneous liver biopsies)	3627	4275	6 pts (0.17% of pts; 0.14% of bps)	6/? (total no. of pts with malignancies NR)	32 pts[b] (0.75% of all biopsies)	22/3627 (0.51%) requiring admission	15/3627[c] (0.35%)	15/32 (47%; HCC n=5; metastases n=10) 8/15 (53%) bleeding had malignancy
Appelbaum, 2008[20]	Retrospective study (FNAB)	208	208 (408 passes)	0	0	0	10/208 (4.8%) requiring analgetics	0[d]	0 (176 malignant tumors of which 128 metastases)
Thanos, 2005[21]	Prospective study (18-gauge automated biopsy gun	767	(2351 passes)	0	0	0	8/767 (1.0%) had mild pain during biopsy	1[e]	NR (736/767 malignant tumors; of which 193 metastases)
Terjung, 2003[22]	Retrospective review of records	574	629	3/629 (0.48%)	0	10/629 (1.6%)	NR	72/629[f] (11.4%)	7/79 (8.9%; HCC n=7/26, metastases n=0/53)
McGill, 1990[23]	Prospective recorded data	9212	9212	10/9212 (0.11%)	7/1766 (0.4%)	22/9212 (0.24%) hemorrhage	NR	22/9212 (0.24%) significant bleeding	0.57% non fatal hemorrhage in cancer pts (2-6 x higher risk of bleeding than without cancer)
Piccinino, 1986[24]	Retrospective multicenter study	NR	68276	6 pts	3 pts (total no. of pts with malignancy NR)	NR	NR	42/68276 (0.06%)	5/1755 (0.28%)

NR = not reported; [a] five pts died due to massive bleeding and one due to aspiration pneumonia and congestive heart failure; [b] 15/32 (47%) had malignancy; [c] 8/15 (53%) had malignancy; [d] not checked for subclinical hemorrhage; [e] one perihepatic hematoma; [f] symptomatic and asymptomatic

18.3 Appendix C: Response evaluation for patients with bone only disease

Lytic bone lesions may be evaluable and should be evaluated using RECIST if significant soft tissue components are available. All evaluations based on tumor markers or circulating tumor cells will be called progression if more than 25% increase has occurred compared to the nadir value, with a mandatory confirmation of the progressive value after at least 4 weeks of additional treatment.

C.1 Response evaluation for breast cancer patients with bone only disease

All breast cancer patients with bone only breast cancer will undergo testing of known serum tumor markers (CA15.3 and others where available) or circulating tumor cells and positive markers will be followed at every disease evaluation time point. Patients that have a baseline CT or MRI will be followed using that modality at the given time points as defined for the treatment in the CPCT-02 Study Manual. Patients with disease only detectable with nuclear bone scans cannot be included due to lack of evaluable disease.

C.2 Response evaluation for prostate cancer patients with bone only disease

All prostate cancer patients with bone only prostate cancer will undergo testing of PSA at every disease evaluation time point. Patients that have a baseline CT or MRI will be followed using that modality at the given time points as defined for the treatment in the CPCT-02 Study Manual. Patients with disease only detectable with nuclear bone scans cannot be included due to lack of evaluable disease.

C.3 Response evaluation for all other tumor types with bone only disease

All patients with bone only cancer outside of breast cancer and prostate cancer that have a baseline CT or MRI will be followed using that modality at the given time points as defined for the treatment in the CPCT-02 Study Manual. Patients with disease only detectable with nuclear bone scans cannot be included due to lack of evaluable disease.

References

(1) Hait WN. Anticancer drug development: the grand challenges. Nat Rev Drug Discov 2010; 9(4):253-254.

(2) Bang YJ, Van CE, Feyereislova A, Chung HC, Shen L, Sawaki A et al. Trastuzumab in combination with chemotherapy versus chemotherapy alone for treatment of HER2-positive advanced gastric or gastro-oesophageal junction cancer (ToGA): a phase 3, open-label, randomised controlled trial. Lancet 2010; 376(9742):687-697.

(3) Piccart-Gebhart MJ, Procter M, Leyland-Jones B, Goldhirsch A, Untch M, Smith I et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005; 353(16):1659-1672.

(4) Romond EH, Perez EA, Bryant J, Suman VJ, Geyer CE, Jr., Davidson NE et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005; 353(16):1673-1684.

(5) van Oosterom AT, Judson IR, Verweij J, Stroobants S, Dumez H, Donato di PE et al. Update of phase I study of imatinib (STI571) in advanced soft tissue sarcomas and gastrointestinal stromal tumors: a report of the EORTC Soft Tissue and Bone Sarcoma Group. Eur J Cancer 2002; 38 Suppl 5:S83-S87.

(6) Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM et al. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. N Engl J Med 2001; 344(14):1031-1037.

(7) Kantarjian H, Sawyers C, Hochhaus A, Guilhot F, Schiffer C, Gambacorti-Passerini C et al. Hematologic and cytogenetic responses to imatinib mesylate in chronic myelogenous leukemia. N Engl J Med 2002; 346(9):645-652.

(8) Fong PC, Yap TA, Boss DS, Carden CP, Mergui-Roelvink M, Gourley C et al. Poly(ADP)-ribose polymerase inhibition: frequent durable responses in BRCA carrier ovarian cancer correlating with platinum-free interval. J Clin Oncol 2010; 28(15):2512-2519.

(9) Fong PC, Boss DS, Yap TA, Tutt A, Wu P, Mergui-Roelvink M et al. Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers. N Engl J Med 2009; 361(2):123-134.

(10) Flaherty KT, Puzanov I, Kim KB, Ribas A, McArthur GA, Sosman JA et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med 2010; 363(9):809-819.

(11) Hanahan D, Weinberg RA. The Hallmarks of Cancer. Cell 2000; 100(1):57-70.

(12) Aparicio SA, Huntsman DG. Does massively parallel DNA resequencing signify the end of histopathology as we know it? J Pathol 2010; 220(2):307-315.

(13) Shah SP, Morin RD, Khattra J, Prentice L, Pugh T, Burleigh A et al. Mutational evolution in a lobular breast tumour profiled at single nucleotide resolution. Nature 2009; 461(7265):809-813.

(14) Ding L, Ellis MJ, Li S, Larson DE, Chen K, Wallis JW et al. Genome remodelling in a basal-like breast cancer metastasis and xenograft. Nature 2010; 464(7291):999-1005.

(15) Yachida S, Jones S, Bozic I, Antal T, Leary R, Fu B et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. Nature 2010; 467(7319):1114-1117.

(16) Campbell PJ, Yachida S, Mudie LJ, Stephens PJ, Pleasance ED, Stebbings LA et al. The patterns and dynamics of genomic instability in metastatic pancreatic cancer. Nature 2010; 467(7319):1109-1113.

(17) Peppercorn J, Shapira I, Collyar D, Deshields T, Lin N, Krop I et al. Ethics of mandatory research biopsy for correlative end points within clinical trials in oncology. J Clin Oncol 2010; 28(15):2635-2640.

(18) Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer 2009; 45(2):228-247.

(19) Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. Liver Int 2008; 28(5):705-712.

(20) Appelbaum L, Kane RA, Kruskal JB, Romero J, Sosna J. Focal hepatic lesions: US-guided biopsy--lessons from review of cytologic and pathologic examination results. Radiology 2009; 250(2):453-458.

(21) Thanos L, Zormpala A, Papaioannou G, Malagari K, Brountzos E, Kelekis D. Safety and efficacy of percutaneous CT-guided liver biopsy using an 18-gauge automated needle. Eur J Intern Med 2005; 16(8):571-574.

(22) Terjung B, Lemnitzer I, Dumoulin FL, Effenberger W, Brackmann HH, Sauerbruch T et al. Bleeding complications after percutaneous liver biopsy. An analysis of risk factors. Digestion 2003; 67(3):138-145.

(23) McGill DB, Rakela J, Zinsmeister AR, Ott BJ. A 21-year experience with major hemorrhage after percutaneous liver biopsy. Gastroenterology 1990; 99(5):1396-1400.

(24) Piccinino F, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. J Hepatol 1986; 2(2):165-173.