ctDNA for Monitoring Treatment Response (ctMoniTR) Project

Comprehensive Proposal

Contents

Background and Rationale

The use of ctDNA to monitor response to therapies is a crucial and timely area of investigation, but it has been particularly challenging to assess its role as a monitoring tool due to variability in the way ctDNA assessment has been designed into clinical trials, the various collection methods used and the way ctDNA changes are reported by different ctDNA assays.

In November 2018, at the Friends of Cancer Research Annual Meeting, a group of experts in the field of liquid biopsies discussed the role of ctDNA for monitoring a patient's tumor response, and developed a white paper that laid the rationale for the use of ctDNA as a feasible and less-invasive method to assess treatment response in patients with cancer and proposed a framework for a uniform collection method.

Building on the white paper and the feedback received at the Annual Meeting, *Friends* developed a proposal for the creation of a pilot project that would assess the feasibility of using ctDNA to monitor treatment response. In February of 2019, *Friends* hosted a full-day roundtable meeting to review this proposal with a multi-stakeholder group comprised of more than a dozen pharmaceutical companies, diagnostic companies, non-profit organizations and members of the U.S. FDA. Practical approaches and implementation of ctDNA as a monitoring tool for treatment response in new and ongoing clinical trials were discussed, as well as how this approach will help answer a relevant, but yet unanswered scientific question: **do changes in ctDNA levels accurately reflect the therapeutic effect of cancer therapies?** As we heard from key opinion leaders, as well as various stakeholders and experts, including biostatisticians and diagnostic developers, we started to identify the gaps our proposed project could fill and the available resources that we could take advantage of to advance the field of liquid biopsies.

The current revised and refined proposal presents a multi-phased pilot project named ctDNA for Monitoring Treatment Response (ctMoniTR), which will 1) harness the wealth of previously collected/published datasets and 2) propose the creation of a prospectively collected harmonized dataset to examine the role of ctDNA as a tool for monitoring treatment response. This collaborative research initiative will help determine the reproducibility of changes in ctDNA across different research programs and classes of drugs, thereby strengthening the evidence for ctDNA use as a monitoring tool independent of any single program/drug.

This document proposes several data scenarios and defines what type of data will be needed to respond several key methodological and knowledge questions. Further, by using a common methodology within different clinical trial subsets, this partnership will generate large scale data faster than if ctDNA continues to be collected in independent, unaligned studies. The document proposes alignment of plasma collection methodologies and suggests uniform approaches for reporting relative directional changes in ctDNA measured by NGS panels. The results of this project will help inform the future application and use of ctDNA in new drug development programs and will generate the much-needed foundation for regulatory evaluation of ctDNA as a monitoring tool.

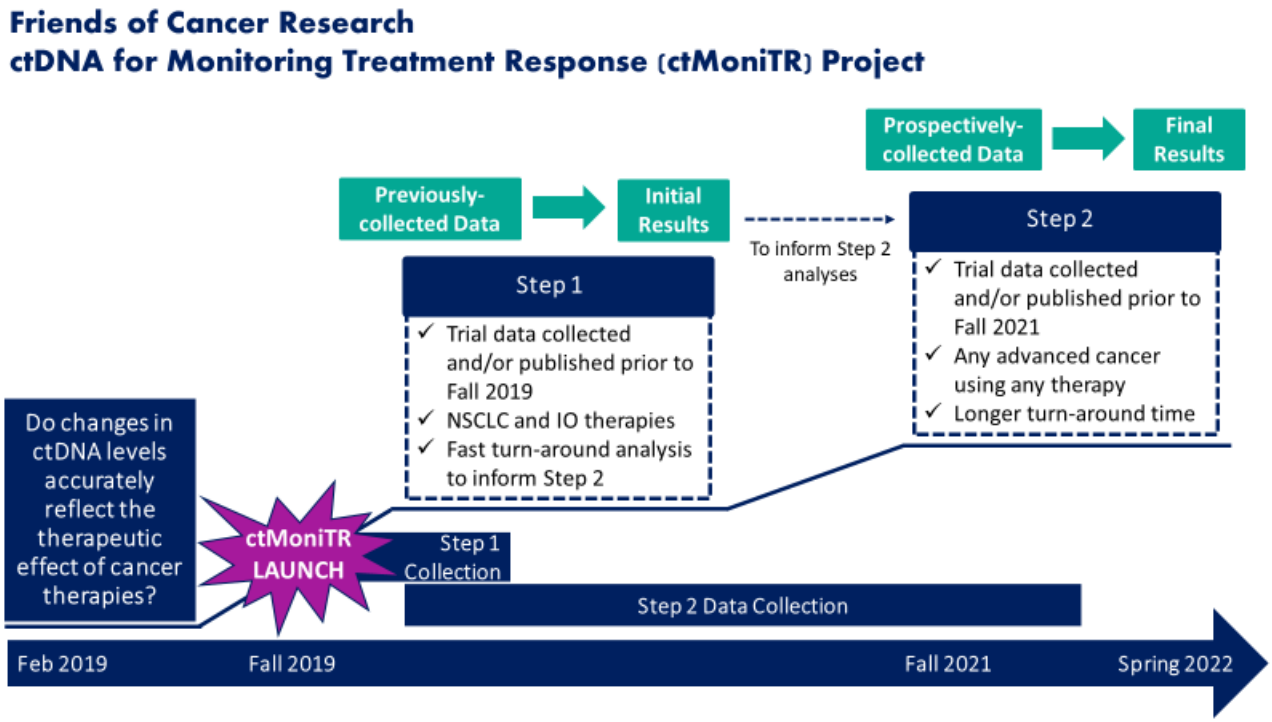

Figure 1: Friends of Cancer Research ctDNA Monitoring Pilot Project (ctMoniTR) Schema

The project (Figure 2) consists of two phases. **Phase 1** involves the use of datasets from trials where quantitative and qualitative ctDNA data has been generated from NGS ctDNA assays, where treatment response (ORR, PFS and/or OS) has been published prior to September 2019. At a minimum, we will focus on lung cancer (NSCLC) and immune checkpoint inhibitors where there is the most data. Other cancer types could include head & neck cancers and melanoma if sufficient data are available. To establish completeness of the Phase 1 dataset, *Friends* will survey sponsors, ct.gov, the literature and other sources of information. *Friends* will list any data not accessible as part of the project report to evaluate the impact of selection bias.

Selected trials must have collected plasma in a way that largely reflects the collection methods proposed by the ctMoniTR Consortium (Table 1). Selected trials must also have ctDNA data from panel-based NGS ctDNA assays and assess changes in ctDNA in a manner that at least follows the parameters provided in Table 2 for assessing relative changes in ctDNA from baseline. Initial results obtained from phase 1 will inform the analyses conducted in Phase 2.

Phase 2 would consist of a study where participating sponsors implement the uniform plasma collection method and standard practices for data processing and analysis as part of ongoing or planned clinical trials. We would assess the feasibility of first, bringing together data from several clinical trials that are investigating same in-class agents in a specific indication (as in Phase 1), then comparing across trials investigating different drug classes and/or indications, and finally, assessing the reliability of ctDNA as an indicator of drug activity and patient response using a larger sample size. Participation in one phase doesn't preclude participation in the other. As the third-party recipient of data, *Friends* will honor any necessary confidentiality agreement to promote and enable participation in this collaborative initiative.

As part of ctMoniTR, a critical mass of data will be accrued in a harmonized manner. This large dataset will promote a greater understanding on how changes in ctDNA may reflect changes in patient response and help develop the evidence necessary to support regulatory evaluation of ctDNA as a reliable monitoring tool for treatment response.

Uniform Plasma Collection Method

The ctMoniTR Consortium proposes the following plasma collection parameters to seek the uniformization of all material used to assess the reliability of ctDNA as a treatment response monitoring tool. These recommendations are based on the pre-analytic cfDNA minimal technical data elements (MDTEs) proposed by the Blood Profiling Atlas in Cancer (BloodPAC) Consortium.

Table 1: Uniform Plasma Collection Method for the Friends of Cancer Research ctMoniTR

Parameter*	**Proposed in ctMoniTR**
Timepoints	1. Baseline: Pre-treatment collection 2. Early collection (after 2-4 weeks or at C2D1) 3. Intermediate collection (at 10-18 weeks, planned around scan timing or treatment cycle) 4. Last collection (at end of treatment [EoT] or at/after progression-prior to next therapy)
Tube types	Streck or EDTA tubes.
Material collected	Plasma
Blood fractionation method	Centrifugation
Number of centrifugation spins	Double spin
Time to fractionation	Within 6 hours of blood draw if using EDTA tubes Within 7 days if using Streck tubes
Holding temperature	4°C for isolated DNA Room temperature for spun plasma in blood tubes
Storage temperature	-20°C if short term storage -80°C if long term storage

DNA quantification	Fluorometric method (e.g. Qubit)

* Collection parameters fall in line with the Blood Profiling Atlas in Cancer (BloodPAC) Consortium Pre-Analytic cfDNA Minimal Technical Data Elements (MDTEs)

Guideline for Alignment of Relative ctDNA Measurements using NGS Panels and Description of Directional Changes in ctDNA

Based on peer-reviewed published work that examined the role of ctDNA in monitoring response to the immune checkpoint inhibitor, durvalumab, in three different lung and bladder cancer trials (Raja et al. 2018 Clin Cancer Res), and the engagement and discussion with representatives from four leading liquid biopsy panel developers, a guideline for alignment of ctDNA measurements using NGS panels was proposed (Table 2).

Table 2: Parameters For ctDNA Measurements Using NGS Panels and Output

Parameter	**Proposed in ctMoniTR**
Technology for ctDNA assessment	NGS gene panel
Min VAF	0.40%
Limit of detection (>95%)	0.20-0.25%
Inclusion of alterations	Somatic SNVs, indels, amplifications and fusions
Type of variants included	Non-synonymous and synonymous variants
Variants not detected at baseline but detected at later timepoints	Baseline VAF will be set to 0
Primary estimate	Mean VAF % & difference between timepoint X and baseline (fold-change)
Reportable output	Aggregate of direction and magnitude (e.g. At 10 weeks from first infusion, a 3-fold reduction from baseline was observed in the responders group)

Legend: ctDNA, circulating tumor DNA; indels, short insertions and deletions; NGS, next generation sequencing; SNVs, single nucleotide variants; VAF, variant allele fraction.

Statistical Analyses Approaches for the ctDNA Monitoring Treatment Response (ctMoniTR) Project

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Following the roundtable discussion that *Friends* hosted in February 2019, where the ctDNA pilot project was initially proposed, *Friends* had several discussions with numerous sponsors and experts in the field of

liquid biopsies. These conversations further refined the pilot approach and yielded informal commitments from many parties and interest from the FDA. In order to launch a formal project, *Friends* now seeks feedback on a range of options for implementation. This document provides a high-level overview of the options and considerations for each.

By September 2019, *Friends* seeks indicative commitment from each individual sponsor, and following a one-day workshop in the Fall of 2019, *Friends* will seek formal commitment with the intent to launch the project publicly.

All contributing organizations will be considered members of the ctMoniTR Consortium. They will participate in the creation of the analysis plan and will receive the draft report for review and comment. Prior to the report's finalization, *Friends* will hold a workshop to ensure all ctMoniTR members are part of the interpretation of the results. *Friends* would like the results to be presented at the timeliest scientific conference post completion of each phase of the work alongside a publication in a peer reviewed journal. The project will include representatives of the U.S. FDA and potentially worldwide health authorities at regular intervals.

In this document, we describe several scenarios for data collection and the associated strategies for analyses. We also list the hypotheses to be evaluated and the degree to which each scenario informs the hypotheses. We encourage members of the ctMoniTR Consortium to review the scenarios, determine what hypotheses you are interested in answering as part of this Project and as a result, determine what level of data you would be willing to provide for this Project.

Scenario A (Summary Data)

Brief Description

For scenario A, trial summary data (SD) is provided to *Friends* who will work with a qualified statistician. In this scenario, each sponsor provides summary data of ctDNA (as an aggregate of direction and magnitude) and the outcomes for each of their trials. *Friends* will both plot the individual study SD in a harmonized fashion and aggregate the SD across all trials to perform the overall evaluation.

General Data Requirements

Sponsors will have to provide general trial information on:

- treatment regimens and their administration
- design variables such as patient inclusion/exclusion characteristics, …
- specifics of the ctDNA assay used including variant calling parameters
- rescue and crossover treatment permissibility
- the trial's final statistical analysis plan
- the trial synopsis or protocol and all amendments

Sponsors will have to provide data on each treatment arm including:

- ctDNA assay data
- outcomes data
- select patient level covariates prior to randomization/entry into the trial

- at each timepoint: number of patients alive, number of patients withdrawn, number of patients lost to follow up, number of patients crossed-over or otherwise 'rescued'
- *optional: other post-randomization biomarker responses either previously studied and discounted or under active evaluation for their predictive value*

Specific Data Requirements

In scenario A sponsors will provide summary data as follows for each treatment arm and timepoint. The sponsors do not perform any analyses, these are performed by *Friends*'s statistician.

- ctDNA assay data: mean/median and 95% CI for data in original scale, fold-change from baseline, log transformed and percent change from baseline.
- tertile and quintile of above (only for trials with a minimum of 45/arm and 75 patients/arm respectively)
- Two response definitions will be applied: CR&PR vs SD&PD and CR&PR&SD vs PD.
- Tumor size outcomes data presented as <0, <10, <20, <30 and >30 response (associated with each summary measure of ctDNA in the first two bullets).
- Sponsor will also provide the differential between treatment arms for the above.

Proposed Analyses

Proposed Descriptive Analyses

- ctDNA over time with an overlay of the outcome variables on second Y-axis. Both treatments will be represented separately on the same graph. Another set of graphs will show the differential between treatments.
- Descriptive plots with various outcome variable response definitions.
- For each post-randomization timepoint, ctDNA tertile and quintile measurements on x-axis vs each associated response. Both treatments will be represented separately on the same graph. Another set of graphs will have the differential between treatment arms. An appropriate measure of monotonicity will be derived.
- Other descriptive graphical representations of the data will be proposed by the ctMoniTR Consortium

Proposed Aggregate Analysis

- Descriptive graphical representation over time for ctDNA vs outcomes measure as above, sized by amount of data in each trial.
- Appropriate meta-analysis methods to establish the ctDNA threshold that is associated with various response/non-response definitions. Associated forest plots.
- An appropriate weighted regression analysis, with and without pre-randomization covariates. The level of association between the outcome measure and ctDNA will be compared to that of the covariates.
- Other analyses may be proposed by members of ctMoniTR Consortium.

This can be applied to any other biomarker for the purposes of internal benchmarking.

Hypotheses under evaluation

Hypotheses are separated into methodology questions which are foundational to the subsequent hypotheses related to ctDNA, and the knowledge hypotheses which evaluate the utility of ctDNA for patient care, use in drug development and its regulatory utility.

A. Methodology Hypotheses (MH) for evaluation:

1. Choice of scale & measurement for ctDNA: There are number of options to represent ctDNA data from the various assays used for this pilot project. Options include nonparametric measures such as the median, tertile values or quintile values. Equally possible, though less likely is the mean from original scale. Other options, such as log transformation can readily address influence of outliers, and percentage change from baseline may allow some degree of harmonization across assays which enables aggregation of data across trials.
2. Rank based analysis approaches vs direct use of raw or log transformed ctDNA values.
3. How are responders best defined in this setting? E.g. should stable disease be considered a non-response in this setting? Should non-responders be assessed at first scan or second scan or end of treatment? Should responders be defined by best reduction of 10, 20% or 30% and so on?

B. Knowledge Hypotheses (KH) for evaluation:

1. Population distribution of ctDNA at baseline, post treatment over time, and in subgroups of interest. This is a basic description of the data.
2. Relationship of ctDNA with patient characteristics at baseline
    1. Are changes in ctDNA associated with a selection of baseline covariates? Answers basic correlation questions as we begin to investigate what the degree of association might be between the ctDNA data and other prognostic factors.
    2. Can other patient characteristics predict ctDNA changes? (reverse question to that above)
3. Relationship of ctDNA to tumor response, PFS and/or OS
    1. Are changes in ctDNA predictive of tumor response, PFS and/or OS?
    2. What is the time course of the association?
    3. How does the predictive value of ctDNA compare to that of other baseline covariates?
    4. Is there an interaction between ctDNA and select subgroups such as PD-L1 status? For example, can we identify PD-L1 negative patients who could benefit from PD-L1/PD-1 treatment by their ctDNA response?
4. If an association exists, what is the threshold of response in ctDNA which differentiates responders from non-responders in the overall population, subgroups of interest, etc.?

A summary of these hypotheses and how well these can be addressed in this scenario is provided on Table 3:

Table 3. Summary of Hypotheses Under Scenario A

Methodology Hypotheses	Analysis – Scenario A	Notes
MH1. Choice of scale & measurement for ctDNA	Descriptive	Adequate, all options to be defined ahead of time to avoid iteration back to sponsors

MH2. Rank based analysis approaches vs direct use of raw or log transformed ctDNA values	Descriptive	Limited, all options to be defined ahead of time to avoid iteration back to sponsors
MH3. How are responders best defined in this setting?	Descriptive	Adequate, all options to be defined ahead of time to avoid iteration back to sponsors
Knowledge Hypotheses* **	**Analysis – Scenario A**	**Notes**
KH1. Population distribution of ctDNA at baseline, post treatment over time, and in subgroups of interest	Descriptive	Limited, based on summary data, limited evaluation of outlier and other distribution features
KH2.1 Are changes in ctDNA associated with select baseline covariates?	Descriptive	Limited since all data are provided in summary form
KH2.2 Can other patient characteristics predict ctDNA changes?	Descriptive	Limited since all data are provided in summary form
KH3.1 Are changes in ctDNA predictive of tumor response, PFS and/or OS?	Graphical and Meta-regression without covariates	Limited as we cannot assess relative to timing of response
KH3.2 What is the time course of the association?	None	Cannot be addressed
KH3.3 How does the predictive value of ctDNA compare to that of other baseline covariates	Meta-regression with covariates	Limited
KH3.4 Is there an interaction between ctDNA and select subgroups such as PDL1 status. For example, can we identify PDL1 negative patients who benefit from PDL1/PD1 treatment by their ctDNA response?	Meta-Analytic Techniques	Limited
KH4 If an association exists, what is the threshold of response in ctDNA which differentiates responders from non-responders in the overall population, subgroups of interest etc.	Forest plots of measures of ctDNA vs various responder definitions	Adequate

*** Importantly, missing data, censoring cannot be addressed in this setting.

Taken as a group, the answers the questions above would greatly advance patient care. Many factors influence the choice and the sequence of the hypotheses chosen for evaluation. *Friends* is committed to a practical, transparent and scientifically rigorous evaluation of the hypotheses ultimately selected by the members of the ctMoniTR Consortium.

Limitations

While this scenario certainly provides adequate information on a number of hypotheses of value to the oncology community, it has some important limitations. Some of these are inherent to the nature of the data. Other may be addressable with input from the sponsor organizations.

1. Cannot pair ctDNA data and outcomes from individual patients leaving out some important information such as individual longitudinal patterns (e.g. lag between ctDNA response and outcome, missing data and censoring.)
2. Patient level covariates are included in a limited fashion, there may not be enough commonality and harmony in this retrospective set of trials (may be addressable in prospective phase of the initiative)
3. All response definition and ctDNA measurements must be pre-defined ahead of time. Any new ideas based on evolving scientific information requires another set of data to be produced by each sponsor.
4. No control for Type 1 error across the multiple analyses (addressable. e.g. setting thresholds to be met for any analysis to be retained for inference)
5. No external validation (addressable via Prospective phase)

Given the limitations above, we next describe the additional value derived from individual patient level data under additional scenarios. These have not been part of the conversation in the past, but we would be remiss if we did not bring up how additional data could help answer important question to advance the field.

Additional Scenarios: Scenarios B, C and D

Brief Description

Scenario B [Patient level data sets analyzed by an independent analysis center]

Deidentified individual patient level data (DIPLD) is provided to an independent analysis center (IAC). This center must be judged to be qualified, especially with respect of handling confidential data/information and respecting firewalls for such data/information across sponsors. Each member of the ctMoniTR Consortium will receive a private report with their data 2 months after submission. Each member will also receive the overall report approximately 2 months after the last data set is received. Members of the ctMoniTR Consortium are invited to nominate up to 2 IAC organizations that *Friends* will consider as part of the selection process. Members of the ctMoniTR Consortium will be able to state their requirements for the selection process.

Scenario C [Patient level data analyzed within each sponsor organization; results are aggregated by IAC for the overall analysis]

IPLD analyzed within each organization according to the central analysis plan. Summary results will be provided to the IAC who will perform the check-in evaluation for each sponsor organization and then aggregate all study level results for the overall analysis. The check-in will consist of a verbal interview with

the analysts who will report any variation and subjective decisions they made beyond the central analysis plan.

One important benefit of scenario C is the familiarity of each sponsor with their own trial data which will ensure greater speed. However, hidden sources of inconsistencies across sponsor trials may go un-addressed. Also, while scenario C allows for aggregation of the results of study level analyses conducted on patient level data, it would not allow for larger scale model building at the patient level across all trials which is possible with Scenario B.

Scenario D [hybrid of Scenarios A and B, SD plus approximately half of DIPLD]- Potential compromise

In this scenario, the summary data is provided as in scenario A along with a 'learning' data set of DIPLD selected through appropriate stratified random scheme (smaller trials included in their entirety). This allows a quick view of the viability of the project based on summary data. The learning data set of DIPLD allows evaluation of some of the deeper scientific and regulatory hypotheses from scenarios A. The ctMoniTR Consortium can then develop the validation hypotheses based on the learning data set which will be evaluated with the remaining half of the data. This approach also allows the ctMoniTR Consortium to determine optimal design and data collection in the proposed prospective phase of the project (Phase 2).

General Data Requirements

Sponsors will have to provide general trial information on:

- treatment regimens and their administration
- design variables such as patient inclusion/exclusion characteristics, …
- specifics of the ctDNA assay used including variant calling parameters
- rescue and crossover treatment permissibility
- the trial's final statistical analysis plan
- the trial synopsis or protocol and all amendments

Sponsors will have to provide data on each treatment arm including:

- ctDNA assay data
- outcomes data
- select patient level covariates prior to randomization/entry into the trial
- at each timepoint: number of patients alive, number of patients withdrawn, number of patients lost to follow up, number of patients crossed-over or otherwise 'rescued'
- *optional: other post-randomization biomarker responses either previously studied and discounted or under active evaluation for their predictive value*

Specific Data Requirements

Scenario B

- In this scenario, sponsors will provide DIPLD to the IAC. The data will include ctDNA values at baseline and each subsequent timepoint, response variables at each time point, baseline

covariates of interest, select post-randomization biomarkers, variables describing treatment received and censoring.

Scenario C

- In scenario C, no data will be required, just the results of the analyses conducted within each sponsor organization. The results obtained will be aggregated by IAC for the overall analysis.

Scenario D

- In scenario D, members of the ctMoniTR Consortium commit to Scenario A as described above in terms of data and analyses. In addition, they commit half of DIPLD for trials with more than 100 patients (selected through appropriate stratified random scheme) and all DIPLD data for smaller cohorts. This forms a learning data set to be analyzed as described under scenario A. From this, the ctMoniTR Consortium will generate the hypotheses and analyses for validation with the remaining data and any new data generated from trials.
- With this approach, the collaboration provides the oncology community with short term output based on aggregated summary data from all available relevant trials (within 6 months from project initiation) and, in the event this is promising, the ctMoniTR Project would already be mobilized to proceed to a 2 step learn/confirm approach with patient-level data from the same set of trials (a further 9 months). As an additional feature, this strategy allows us to verify the most important design elements to be harmonized for the prospective set of trials - trials collecting ctDNA under the uniform method of collection - such as the proposed common set of baseline covariates and their definitions, the timepoints for the collection of data, duration of follow up, and any additional post randomization biomarkers or covariates to be collected. An important consideration before adopting this approach would be the amount of available patient level data. To this end, if sponsors wish to pursue this hybrid option, *Friends* will to collect the sample size data to establish viability (or not) of this hybrid approach. The scenario remains speculative until then.

Hypotheses under evaluation

The hypotheses under evaluation for these additional scenarios remain the same as the ones described in scenario A. However, because of the additional data found in DIPLD, several more hypotheses could be answered. Please see Table 4 below for details.

Table 4. Summary of Hypotheses Under Scenario B & C Unless Otherwise Noted

Methodology Hypotheses	**Scenario B & C unless otherwise noted**	**Notes**
MH1. Choice of scale & measurement for ctDNA	Descriptive	Good – allows full optionality
MH2. Can based analysis approaches vs direct use of raw or log transformed ctDNA values	Descriptive/quantitative	Good – allows for all relevant approaches to be performed and statistically compared
MH3. How are responders best defined in this setting?	Descriptive/quantitative	Good – allows for all definitions to be derived and statistically compared
Knowledge Hypotheses	**Scenario B & C unless otherwise noted**	**Notes**
KH1. Population distribution of ctDNA at baseline, post treatment over time, and in subgroups of interest	Descriptive/quantitative	Good - Full evaluation of distributional features given full data

KH2.1 Are changes in ctDNA associated with select baseline covariates?	Descriptive/quantitative	Good – access to full data allows best characterization
KH2.2 Can other patient characteristics predict ctDNA changes?	Descriptive/quantitative	Good – access to full data allows best characterization
KH3.1 Are changes in ctDNA predictive of tumor response, PFS and/or OS?	Categorical analysis of paired patient level data. In either scenario, the analysis stratified by study can be conducted.	Best - Paired Patient outcomes are described as opposed to population summaries
KH3.2 What is the timecourse of the association?	Time varying covariate analysis	Best - Provides definitive parametric form of ctDNA's vs response over time. (**B**) Adequate – (**C**)
KH3.3 How does the predictive value of ctDNA compare to that of other baseline covariates	Proper model building (and machine learning) approaches are possible under scenario B. In scenario C, we are more limited and drawing inference is more challenging	Best - Provides comprehensive modern evaluation of ctDNA predictive value relative to other covariates, accounting properly for patient and trial-based effects. (**B**) Good – (**C**)
KH3.4 Is there an interaction between ctDNA and select subgroups such as PDL1 status. For example, can we identify PDL1 negative patients who benefit from PDL1/PD1 treatment by their ctDNA response?	Statistically valid testing is available under scenario B. In scenario C, the testing will be done in each trial, so overall inference is more challenging but possible using meta-analytic approaches.	Best – (**B**) Good – (**C**)
KH4 If an association exists, what is the threshold of response in ctDNA which differentiates responders from non-responders in the overall population, subgroups of interest etc.	Scenario B allows a single parametric form to emerge and is thus the gold standard	Best – (**B**) Good – (**C**)

Proposed Analyses

- In scenarios B and D, the IAC will conduct all the analyses described in Scenario A.
- In scenario C, we will ask each sponsor to conduct the described analyses within each organization.
- Additionally, because patient-level data will be available, the following will be performed.
    - Box plot or other distribution plot of ctDNA over time by treatment arm separated by response status at all timepoints. **Can be done for B, C and D**

- Each trial data will be plotted with individual patient ctDNA over time, and their response. The treatment arms will be plotted separately. Summary measures of paired treatment effects on ctDNA and on response will also be plotted. **Can be done for B, C and D.**
- Appropriate paired categorization of patients based on their joint ctDNA and outcomes response longitudinally. For each trial, classical multinomial approaches will be applied to evaluate the degree of association between effect on ctDNA and outcomes. Repeat with trial as a stratification factor for overall aggregate measure of degree of association. **Can be done for B, C and D.**
- Regression techniques will be applied to each trial to determine the level of association and parametric form between ctDNA values and outcomes of CR, PR, SD and PD over time. **Can be done for B, C and D.**
- All patient level data will be included in a model building approach which includes ctDNA and other covariates with study as a stratification factor. Classical linear/non-linear mixed effects modelling approaches as well as data mining approaches will be used to determine the optimal set of parameters to predict response. **Can be done in scenario B, and maybe in scenario D, but <u>not</u> in scenario C.**

- ctDNA will be analyzed on the original scale, change from baseline, log scale or percent change from baseline based on the IAC review of the data. In these scenarios, we can also derive overall measure of patient ctDNA response over time such as area under the curve (AUC).
- Timing of various threshold of responses will be incorporated into the analyses. Sponsors are invited to propose analytical approaches for this line of enquiry (e.g. landmark and time varying covariate analyses).

Additional analyses:

1. Scenarios B&C: Analyses which cannot be performed in scenario A:
    - Multinomial assessment of degree of ctDNA effect vs degree of response.
    - Landmark and time varying covariate analyses

2. Scenario B: Analyses which cannot be performed in either scenarios A or B:
    - Establish the definitive parametric form of ctDNA's vs response over time.
    - Comprehensive modern evaluation of ctDNA predictive value relative to other covariates, accounting properly for patient and trial-based effects.

Limitations

Both scenarios B and C offer the established benefits expected from analyses based on patient level data. As such, they certainly provide more information than Scenario A and lend additional credibility through the IAC, these scenarios require more time from all parties involved and most likely, a greater financial commitment. At this point, it is also unclear if the data are sufficient for the model building proposed in scenario B. However, with the hybrid option, or scenario D, an ideal compromise could be had with a combination of summary and patient-level data, where the sponsor gets to decide the size of the subset of patient-level data they wish to contribute.

Summary

Having reviewed the scenarios, we summarize the value of each approach relative to the hypotheses we set out in the table below. Scenario B is without a doubt the most comprehensive scenario, where we

would be able to answer the most questions. Given the complexity and time involved, sponsors may wish to commit to Scenario A at a minimum, and if promising, pursue either B or C immediately thereafter. We believe a good compromise would be Scenario D, where sponsors provide a combination of summary data (as in Scenario A) with some subset of patient-level data (as in Scenario B). In all cases, it would be valuable to **come to an agreement by October 2019** on the design elements of trials in the prospective phase of the project.

*Table 5. Summary Table of all Hypotheses and How Well Scenarios A, B and C Are Able to Answer the Hypotheses**

Methodology Hypotheses	**Scenario A**	**Scenario B**	**Scenario C**
MH1. Choice of scale & measurement for ctDNA	+	++	++
MH2. Can based analysis approaches vs direct use of raw or log transformed ctDNA values	↔	++	++
MH3. How are responders best defined in this setting?	+	++	++
Knowledge Hypotheses			
KH1. Population distribution of ctDNA at baseline, post treatment over time, and in subgroups of interest	↔	++	++
KH2.1 Are changes in ctDNA associated with select baseline covariates?	↔	++	++
KH2.2 Can other patient characteristics predict ctDNA changes?	↔	++	++
KH3.1 Are changes in ctDNA predictive of tumor response, PFS and/or OS?	+	+++	+++
KH3.2 What is the timecourse of the association?	N/A	+++	+
KH3.3 How does the predictive value of ctDNA compare to that of other baseline covariates	↔	+++	++
KH3.4 Is there an interaction between ctDNA and select subgroups such as PDL1 status. For example, can we identify PDL1 negative patients who benefit from PDL1/PD1 treatment by their ctDNA response?	↔	+++	++
KH4 If an association exists, what is the threshold of response in ctDNA which differentiates responders from non-responders in the overall population, subgroups of interest etc.	↔	+++	++
AVAILABILITY OF RESULTS BY JUNE 2020	YES, likely earlier	Partial, completion expected December 2020	Partial, completion expected September 2020

*Since Scenario D is a combination of Scenario A and Scenario B, data from this scenario would address well all hypotheses.

Legend

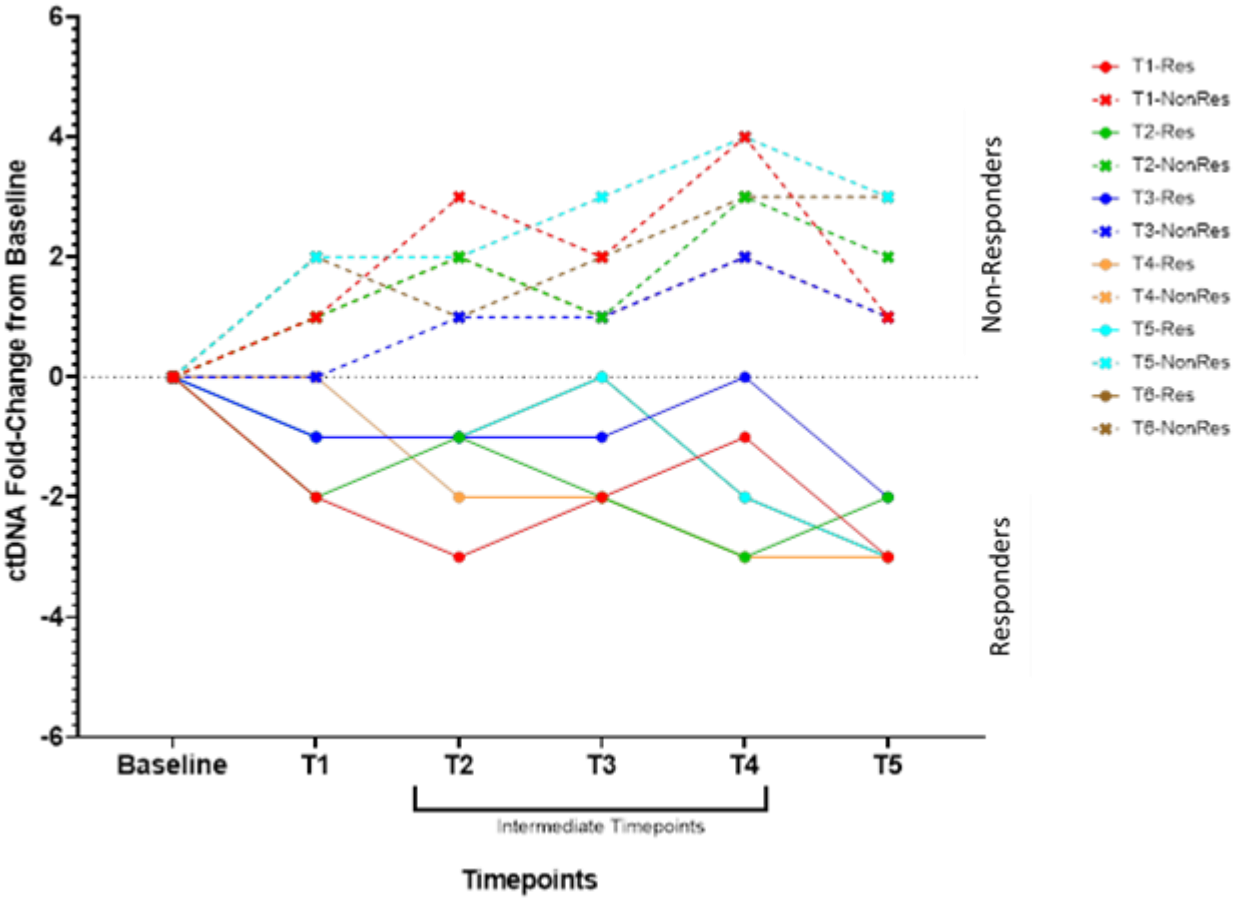
N/A	Not Addressed
↔	Limited
+	Adequate
++	Good
+++	Best

APPENDIX

Appendix Table 1: Mock Table Example for Phase 1 Data Collection

Trial	Sponsor	Indication	Drug Class	ctDNA assay	Patient Response	N	Baseline	T1	T2	T3	T4	T5
aNSCLC (all lines, all stages)												
Trial 1	Sponsor 1	aNSCLC	ICI	Guardant Health	Responders	30	**0**	**-2**	**-3**	**-2**	**-1**	**-3**
				Guardant360	Non-responders	30	**0**	**1**	**3**	**2**	**4**	**1**
Trial 2	Sponsor 2	aNSCLC	ICI	FMI	Responders	22	**0**	**-2**	**-1**	**-2**	**-3**	**-2**
				FoundationACT®	Non-responders	20	**0**	**1**	**2**	**1**	**3**	**2**
Trial 3	Sponsor 1	aNSCLC	ICI	Guardant Health	Responders	55	**0**	**-1**	**-1**	**-1**	**0**	**-2**
				Guardant360	Non-responders	21	**0**	**0**	**1**	**1**	**2**	**1**
Trial 4	Sponsor 1	aNSCLC	ICI	Guardant Health	Responders	23	**0**	**0**	**-2**	**-2**	**-3**	**-3**
				Guardant360	Non-responders	14	**0**	**1**	**2**	**1**	**2**	**1**
Trial 5	Sponsor 3	aNSCLC	ICI	Illumina	Responders	65	**0**	**-1**	**-1**	**0**	**-2**	**-3**
				TSO500	Non-responders	32	**0**	**2**	**2**	**3**	**4**	**3**
Trial 6	Sponson 3	aNSCLC	ICI	PGDx	Responders	12	**0**	**-1**	**-1**	**0**	**-2**	**-3**
				PlasmaSELECT	Non-responders	17	**0**	**2**	**1**	**2**	**3**	**3**

Changes in ctDNA are reported as fold-change from baseline. Legend: aNSCLC, advanced non-small cell lung cancer; ctDNA, circulating tumor DNA; FMI, Foundation Medicine Inc.; ICI, immune checkpoint inhibitors; N, number of patients per response group; PGDx, Personal Genome Diagnostics; T1-T5, timepoints 1-5.



Appendix Figure 1. Conceptual Mock Plot Depicting ctDNA Fold-Change from Baseline for Example for Phase 1 Collection (Appendix Table 1)