FRIENDS of CANCER RESEARCH

A Friends of Cancer Research White Paper

EXPLORING THE USE OF CIRCULATING TUMOR DNA AS A MONITORING TOOL FOR DRUG DEVELOPMENT

INTRODUCTION

*Cell-free DNA (cfDNA)**, defined as free extra-nucleic acid circulating in plasma, was first described in the blood of healthy and diseased individuals in 1948.[1] Most cfDNA in blood is derived from ruptured nonmalignant cells arising from normal physiological tissue remodeling events and originates from the germline. However, in patients with cancer, a fraction of this cfDNA is made up of nucleic acids that are shed from primary or metastatic lesions undergoing tumor cell apoptosis and necrosis and are referred to as *circulating tumor DNA* (ctDNA). ctDNA is composed of small fragments of nucleic acid that are not associated with cells or cell fragments, thus differentiating it from circulating tumor cells (CTCs).

The greatest proportion of DNA fragments in circulation measure between 180-200 nucleotides in size, suggesting they are a result of cellular apoptosis; however, much smaller fragments have been reported in some tumor types, such as hepatocellular carcinoma, as well as much larger fragments consisting of thousands of base pairs that may be a result of tumor necrosis.[2] The amount of ctDNA in circulation is very small ranging between <0.1-10% of total cfDNA detectable in human blood. This value varies according to tumor burden or size, inflammatory status, cellular turnover, and proximity of cancer cells to blood vessels.[3]

The ability to detect small amounts of ctDNA in fluids has given rise to the use of liquid biopsies, a minimally invasive test done on a blood sample, or other fluids, that provide an alternative to surgical biopsies of solid tissues.[4] The recent development of large-scale genomics and bioinformatics approaches has facilitated the use of highly sensitive molecular assays that can detect tumor-specific alterations present in at least 5% of the cells analyzed and at

*Terms in italics are defined at the end of this documentin the "List of Definitions."

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Friends of Cancer Research drives collaboration among partners from every healthcare sector to power advances in science, policy, and regulation that speed life-saving treatments to patients.

frequencies as low as 0.05%.[5] Classical methods for ctDNA analysis include hotspot assays that detect specific known somatic variants at very low levels found in a single gene or small number of genes, and typically use polymerase chain reaction (PCR)-based strategies such as *digital droplet PCR (ddPCR)*, or *real-time PCR (RT-PCR)*. More recent strategies use *next-generation sequencing (NGS)* approaches for the detection of somatic and germline (heritable) variants in more than one gene target and are capable of detecting a larger number of variants in multiple genes. Deep sequencing can typically detect tumor-specific alterations in the whole genome or exome, and most recently, in gene panels that have been especially designed to incorporate relevant genes associated to cancer growth and progression. The most common ctDNA genomic alterations identified include point mutations, deletions, amplifications and translocations, and gene fusions.[5,6] Measuring these alterations in the ctDNA isolated from cancer patients' blood can considerably facilitate the clinical management of patients diagnosed with blood cancers and solid tumors. Although assessing disease burden using blood samples is already a common practice for patients with blood malignancies, investigating blood for traces of solid tumor cells and DNA is a more recent practice, and has the potential to facilitate clinical cancer care and benefit more patients.

First, because drawing blood for a *liquid biopsy* is minimally invasive and significantly less risky than conducting a tissue biopsy, especially for tumors that are not easily accessible, using *ctDNA assays* to conduct repeated assessments that monitor a patient's tumor response over time poses less risk to the patient. Moreover, because the test can be conducted at a central site, patients don't need access to technical molecular pathology labs, which are rarely found in the community setting. Analysis of ctDNA is more convenient and logistically feasible than traditional biopsies, and it is a tool that can democratize access to powerful diagnostics and targeted therapies regardless of where a patient receives care.

The assessment of ctDNA requires powerful technology that is highly sensitive and dynamic and enables the detection of very small amounts of tumor DNA from very early to well advanced stages of disease. Additionally, the multiplex assays used in ctDNA analyses capture a broad array of somatic, or tumor-derived, genetic alterations found in numerous genes from ctDNA in blood (*i.e., genotyping*).

Leveraging the advent of new technologies with these remarkable features, ctDNA may be used in a way that goes beyond simply identifying the presence or absence of tumor DNA in blood but could be potentially used for (1) *cancer detection*, (2) prognosis determination, and (3) molecular characterization of a patient's tumor.

As a powerful cancer screening tool, ctDNA could be used for early cancer detection. This could mean detecting cancer prior to a cancer diagnosis in an asymptomatic population, or detecting early recurrence, or the degree or burden of disease in patients that have already been diagnosed with cancer.[5,7] ctDNA could also help assess patient prognosis. This could mean catego-

rizing patients into different risk groups by examining the presence of specific somatic genetic alterations associated to patient outcomes.[2,8-11] Lastly, identifying somatic genetic alterations in ctDNA would enable the molecular characterization of the tumor, which could guide targeted therapy selection and identify potential mechanisms of tumor resistance.[12-13]

Although using ctDNA for cancer detection, prognosis determination, and molecular characterization of a patient's tumor are very important and becoming more common in clinical practice, this white paper will focus on recommending best practices for the use of ctDNA for disease *monitoring* in cancer patients and will investigate the feasibility of operationalizing this tool in drug development. Additionally, even though much effort has been given to the definition and study of *minimum residual disease* as a way to monitor disease response and progression in patients with blood cancers, this white paper will concentrate on the use of ctDNA in patients with solid tumors.

Given the rapid advancement of technologies that have promoted the use of ctDNA in drug development and the growing number of studies that seek to use liquid biopsies as a tool to assess tumor response; Friends of Cancer Research (*Friends*) has convened a multi-stakeholder group of experts to examine the state of ctDNA in tumor monitoring, recommend best practices, and propose initiatives that would directly demonstrate how data derived from ctDNA could be used to facilitate cancer drug development.

OBJECTIVES

The objectives of this white paper are to assess the current state of ctDNA as a monitoring tool used to evaluate clinical response through the description of relevant case studies, suggest best practices for the use of ctDNA as a potential monitoring tool for drug development in clinical research, and propose two potential opportunities that promote the operationalization of ctDNA in drug development.

CASE STUDIES

Various studies have investigated the use of cfDNA or ctDNA to monitor tumor response. Many of these studies have been retrospective using previously collected data and consisting of a few samples. The working group identified three prospective clinical trials where serial analyses of cfDNA was used to gain insight into treatment effect (Table 1). These prospective studies demonstrate the diverse ways investigators are using cfDNA to monitor clinical outcomes, highlighting the promising potential of this accessible biomarker in clinical trials, but also unraveling the difficulties that lie in seeking to compare data from all three studies given the different methods, units, and outcomes assessed in each study.

1. **Detection and Dynamic Changes of EGFR Mutations from Circulating Tumor DNA as a Predictor of Survival Outcomes in NSCLC Patients Treated with First-line Intercalated Erlotinib and Chemotherapy, 2015** [14]

Mok and colleagues describe the findings of a multicenter, randomized, placebo-controlled, double-blind, phase III study of intercalated erlotinib or placebo with gemcitabine plus platinum followed by maintenance erlotinib or placebo as first-line treatment in patients with stage IIIB/IV NSCLC (FASTACT-2). The primary objective of this study was to define the diagnostic utility of a RT-PCR based blood test that detects activating mutations in EGFR in cfDNA. The secondary objective was to examine the predictive value of cfDNA EGFR at baseline and the changes in mutation status during therapy in relation to patient outcomes. This study found very high concordance between tissue and blood tests, that EGFR mutation status defined by blood-based cfDNA analysis appears to produce similar results to tissue-based assessment in terms of predicting outcomes, and that dynamic changes in cfDNA EGFR mutation status correlate with disease progression, ORR, and survival.

Blood from 305 patients was extracted according to standard procedures at baseline, at day 1 of cycle 3 (C3, mid-protocol), and at the time of progression, while tumor tissue samples were obtained at initial diagnosis, diagnosis of advanced disease, or biopsy 14 days before first study dose. The cobas 4800 blood test by Roche Molecular Systems Inc. was used to detect 41 different EGFR activating mutations. The number of EGFR mutant copies (copy/mL of blood) were measured across the three timepoints (baseline, C3, and PD) and correlated with ORR, PFS, and OS.

This study found that generally, total EGFR mutation-specific cfDNA levels decreased at C3 and returned at time of PD, which may reflect changes in tumor volume or increased metastases. For patients with detectable EGFR mutations at baseline, ORR was lower in patients whose cfDNA analysis showed detectable EGFR mutations at C3 (mid-protocol) compared with patients whose cfDNA analysis showed undetectable EGFR mutations at C3. Likewise, the PFS and OS of patients whose cfDNA samples remained positive for EGFR mutations at mid-protocol were also lower

Table 1: Case studies and study parameters

Parameters/Study	Mok et al., Clinical Cancer Research, 2015[14]	Yu et al., Clinical Cancer Research, 2017[15]	Raja et al., Clinical Cancer Research, 2018[16]
Histology	Stage IIIB and IV NSCLC	Advanced NSCLC patients with disease progression after EGFR TKI treatment	NSCLC and UC
# of patients	305	93	100 (28 discovery, 72 validation) and 29 (validation) from 2 different studies
Clinical trial	FASTACT-2 study	NCT02113813	ATLANTIC and Study 1108
ctDNA/cfDNA	cfDNA	cfDNA	ctDNA
Technology	Semi-quantitative—Cobas 4800 blood test (RT-PCR)	Quantitative—BEAMing PCR	Quantitative—NGS, targeted panel (Guardant 360)
Gene	EGFR	EGFR	Gene panel (73 genes)
Units	Copy/mL	% mutant EGFR cfDNA	Mean VAF
Timepoints	Baseline, cycle 3 (~12 weeks) and progression (PD)	Baseline, cycle 2	Baseline and 6 weeks-prior to 4th treatment
Median follow up time	Not specified	Not specified	Ranged between 9-15 months depending on study
Drug(s) being tested	Erlotinib (after gemcitabine/platinum)	ASP8273 (3rd generation EGFR TKI)	Durvalumab (anti PD-L1)
Clinical Response/ Outcome	ORR, PFS, OS	ORR	Tumor volume, PFS, OS
Tube	"collected according to standard procedures"	n/a	K2-EDTA
Timing of processing	"collected according to standard procedures"	n/a	n/a

Abbreviations: cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; EDTA, ethylenediaminetetraacetic acid; *EGFR*, epidermal growth factor receptor; NGS, next generation sequencing; NSCLC, non-small cell lung cancer; ORR, objective response rate; OS, overall survival; PD, progressive disease; PD-L1, programmed death-ligand 1; PFS, progression free survival; RT-PCR, real time- polymerase chain reaction; TKI, tyrosine kinase inhibitor; UC, urothelial carcinoma; VAF, variant allele fraction.

than in patients whose cfDNA samples became negative for EGFR mutations.

Authors concluded that assessing EGFR mutation status mid-protocol, in this case at C3, approximately 12 weeks after the start of the first study dose, may predict clinical outcomes and that the serial quantitative measurement of EGFR cfDNA could serve to assess tumor progression. Moreover, because of the good correlation between tumor and blood tests, the authors identified cfDNA EGFR mutation analysis as a potential reliable alternative method for patients from whom a tumor tissue sample cannot be obtained.

2. **A phase 1, dose-escalation/response-expansion study of oral ASP8273 in patients with non-1 small cell lung cancers with epidermal growth factor receptor mutations, 2017[15]**

Yu and colleagues describe the results of a prospective, open-label, multicenter dose escalation phase I study (NCT02113813) testing the third-generation EGFR TKI, ASP8273 in patients with advanced NSCLC harboring EGFR activating mutations and previous EGFR TKI treatment. Exploratory endpoints of this study included the evaluation of potential biomarkers in cfDNA and their association with treatment effects. This study found for patients who achieved partial response and stable disease as best overall response, EGFR activating and T790M mutations in cfDNA were generally reduced to near or below level of detection after 1 cycle of treatment. Additionally, in patients who developed acquired resistance to ASP8273, EGFR activating and T790M mutations reemerged in the plasma of 5 out of 9 patients.

110 patients from the study met the criteria for the study and were assigned to dose-escalation cohorts where ASP8273 was administered orally in a single-dose period lasting 2 days and followed by repeat-dose cycles consisting of once-daily treatment over 21 days. Of the 110 patients, 93 were eligible for biomarker analysis of cfDNA, and 46 out of 93 had sufficient plasma samples for longitudinal analysis. Mutations in EGFR were examined in cfDNA isolated from blood serially collected prior to study start and at each treatment cycle, using beads, emulsification, amplification, and magnetics (BEAMing) digital PCR. Additionally, EGFR mutation status was also assessed centrally by RT-PCR. Percentage mutant EGFR cfDNA (%) was observed at baseline and at cycle 2 in patients with EGFR T790M positive metastatic NSCLC treated with ASP8273. Patients were grouped by best response to ASP8273, including partial response, stable disease, or progressive disease.

The authors concluded that the presence of EGFR T790M mutations in cfDNA predicted response to ASP8273 and that using cfDNA to identify mutation patterns of progression throughout treatment, such as the emergence of new mutations in EGFR, or the reemergence of mutations initially identified at baseline may be potentially useful in the clinic. Reductions in EGFR levels in cfDNA were seen across a broad range of doses in this phase I study (100mg-500mg), which suggests activity of the agent at a range of doses. Due to the high concordance observed with tumor tissue, the authors recommended that further studies to understand the relationship between cfDNA and tumor burden, as well as other clinical parameters, be conducted.

3. ctDNA changes in advanced lung and bladder cancer patients receiving PD-L1 inhibitor (durvalumab) as a potential response biomarker, 2018[16]

This study investigated changes in *variant allele frequencies (VAF)* of somatic mutations in ctDNA from the blood of patients with advanced NSCLC and urothelial cancer (UC) and their association with patient outcomes after treatment with PD-L1 inhibitor durvalumab. The study found that a reduction in ctDNA VAF at 6 weeks is associated to tumor shrinkage and improved progression-free and overall survival.

Patient blood was extracted at baseline (pre-dose) and six weeks after the first dose (post-dose). ctDNA was tested using the Guardant 360 gene panel comprising of 73 genes. Somatic variants, including single nucleotide variants (SNVs), insertions/deletions, and fusions were summarized for each patient by calculating the mean allele frequency of all genes with a VAF ≥0.3% at pre-dose. Both synonymous and non-synonymous mutations were included in the VAF calculation. Change in mean VAF was calculated when mean VAF at pre-dose was subtracted from VAF at 6 weeks. Mean VAF was compared across timepoints (pre-dose and post-dose) and correlated with objective response rate (ORR), time on study, tumor volume, and survival (Table 1).

Patients from two different clinical trials were included in this analysis. Study 1108 (NCT01693562) was a phase 1/2, first-in-human, multicenter, open-label dose-escalation, and dose-expansion study. Eligible patients were ≥18 years of age with histologically or cytologically confirmed inoperable or metastatic transitional-cell UC or NSCLC and who had progressed on, been ineligible for, or refused any number of prior therapies. The second clinical trial, ATLANTIC (NCT02087423), was a multicenter, phase 2 open-label study enrolling patients with Stage IIIB/IV NSCLC with disease progression following two or more systemic treatments, including one platinum-based chemotherapy and one tyrosine kinase inhibitor (TKI) for EGFR mut/ALK+ patients.

This study also observed the emergence of new EGFR mutations in patients with progressive disease at week 6. These mutations have been previously associated with resistance to immunotherapies. Thus, the use of liquid biopsies throughout the course of therapy will enable longitudinal monitoring of changes in tumor burden, and the identification of new mutations that are associated with patient outcomes that may facilitate the development of combination therapies in immuno-oncology.

BEST PRACTICES FOR THE USE OF ctDNA AS A POTENTIAL MONITORING TOOL

The case studies described above demonstrate the potential clinical impact ctDNA may have on disease monitoring and the potential utility liquid biopsies may have to help assess drug efficacy early during a clinical trial. Given the convenience of ctDNA analysis and its ability to quantify mutations in ctDNA throughout treatment and identify new mutations that arise during treatment that may confer resistance to ongoing therapies, identifying a consistent way to use ctDNA as a monitoring tool is imperative. Outcomes of studies to date have been variable, and this variability is explained by different technologies used, the lack of standardization, and the absence of prospective clinical and biomarker data.

Drawing from studies performed to date, their methods and the limitations of those methodologies, as well as from a wealth of personal experience, the working group has generated a list of best practices and recommendations that have been classified into the following categories: material collection, detection platform technology, and analysis (Table 2).

While not the primary focus of this white paper, the need for rigorous analytical validation parameters of ctDNA assays should also be acknowledged. Ongoing efforts being led by other organizations, such as the Blood Profiling Atlas in Cancer (BloodPAC), are determining best practice principles for validating liquid biopsy tools for ctDNA assessment.

Generally, prior to using a ctDNA assay as a tool for drug development in a clinical trial, the assay should be analytically validated, and the cutoffs should be pre-specified and locked down. Some of the key analytical studies include, but not limited to, limit of blank (LoB), limit of quantitation (LoQ, only for quantitative assay i.e., the assay has continuous output), limit of detection (LoD), linearity (only for quantitative assays), analytical accuracy, and precision/reproducibility, should be evaluated to establish optimal assay performance. Since these assays will likely have a quantitative output, it is expected that the analytical and clinical studies are consistent with the assay's intended use. In order to report underlying continuous measures (e.g. MAF, bTMB, circulating tumor fraction), analytical validation studies or analyses should be done to demonstrate that those continuous values can be accurately and reliably measured.

Additionally, for monitoring purposes, the proposed assay should continuously assess a subject's status over a period of time or monitor at intermittent times, and the study duration should be long enough to capture the range of variation in the assay measurements and clinical status from the assay's intended use population. The time interval at which the data is collected and how many and how often data points per patient are collected should be clinically acceptable. How change in the assay result or patient clinical status is defined and determined should be clearly prespecified.

Table 2: Best practices for the use of ctDNA in disease monitoring

Best Practice	Recommendations
Material collection	
Timing	1. Collection at cycle 1, day 1 (screening sample may not be representative) 2. Early collection after 2-4 weeks 3. Collection at the time of restaging scans 4. Collection at or after progression (prior to next therapy)
Amount of material	• One 10ml tube is usually adequate for analysis • Recommend collection of a second 10mL tube for future bridging studies • Recommend saving the cell pellet to allow study of white blood cells if needed.
Tube type	• If site has capacity to spin down tubes locally within a few hours after collection, EDTA tubes would be adequate. Otherwise tubes including a DNA stabilization agent (e.g. Streck tubes) are preferred to allow delayed spinning of specimens
Detection platform technology	• Should be able to measure ctDNA changes quantitatively • Recommend quantification of variant allelic fraction, which can be calculated across various assays (e.g. ddPCR, NGS) • Platform should be validated to show optimal commutability against other assays (orthogonal approaches)
Analysis	• Consider calculation of percent change from baseline, similar to approach used for tumor measurements in imaging • Analysis should account for the possibility of mutations derived from clonal hematopoiesis. Sequencing of white blood cells can be useful for distinguishing this

ESTABLISHMENT OF A MULTI-STAKEHOLDER CONSORTIUM TO OPERATIONALIZE ctDNA IN DRUG DEVELOPMENT

As demonstrated by the case studies above, several studies have examined the association between ctDNA and clinical outcomes in patients with advanced cancer. However, different analytical approaches are currently used in each study, which make it challenging to generate broad learnings across cancer types and treatment settings. Through conversations with multiple stakeholders, this working group has identified two potential opportunities to better understand the relationship between changes in ctDNA levels in plasma and treatment outcomes and promote the operationalization of ctDNA in drug development: a prospective collection of ctDNA from ongoing clinical trials, which will implement standard practices for plasma collection and analyses of plasma response, and the collection of existing datasets from past clinical trials and studies from which to learn how to best use ctDNA in drug development.

ctDNA Pilot Project: Monitoring therapeutic effect of immune checkpoint inhibitors

The variability observed across studies and existing datasets demonstrates the need for the prospective validation of ctDNA in rigorous cohorts. Achieving this will require standardization of data processing, collection, and analysis.

There is a need for the development of standard practices that may promote the integration of ctDNA into clinical trials and facilitate the aggregation and analysis of resulting data. Moreover, it is important to understand how optimal, feasible, and reproducible these practices are, and whether the data collected could be easily aggregated from large trial studies.

A unified prospective pilot could allow us to rigorously address a key clinical question: Do changes in ctDNA levels accurately reflect the therapeutic effect of immune checkpoint inhibitors?

To address this important question, this working group proposes the creation of a pilot project where a standardized add-on study framework is adopted for the collection of a core set of ctDNA measurements and clinical endpoints as part of ongoing or new clinical trials.

The pilot project would assess the feasibility of bringing together data from several clinical trials that are investigating same in-class agents in a specific population and determine the minimum amount of data that sponsors would be willing and able to share to evaluate outcomes based on ctDNA measurements.

Table 3 describes a framework proposed by the working group that could be added on to an ongoing trial. This framework outlines a few key elements that will delineate how ctDNA and clinical data could be collected during clinical trials and proposes methods for assessing the correlation between differences in ctDNA dynamics and response. The working group hopes the frame-

Table 3: *Friends* ctDNA pilot project framework

Parameter	Proposed Pilot
Patient population	Patients with advanced/metastatic disease
Population size	As determined by the clinical trial or drug sponsor
Drug class	Immune checkpoint inhibitors
Trial phase	All phases
Technology for ctDNA assessment	ddPCR or NGS gene panel
Minimum Limit of Detection	0.2-0.25% VAF
Test tubes	If site has capacity to spin down tubes locally within a few hours after collection: EDTA. Otherwise tubes including a DNA stabilization agent (e.g. Steck tubes)
Timepoints	1. Collection at cycle 1, day 1 (screening sample may not be representative) 2. Early collection after 2-4 weeks 3. Collection at the time of restaging scans 4. Collection at or after progression (prior to next therapy)
Median follow up	6 months
Diagnostic endpoints	Relative percent change from baseline
Alterations (definition)	Mutations, insertions, deletions, amplifications, and fusions
Clinical endpoints	Raw tumor size/volume, ORR and PFS and/or OS, if applicable (trial dependent)
Adjustment factors	Age, gender, smoking status, baseline ECOG score, previous line of therapy, and histology

work is reasonable and feasible for participating sponsors to readily incorporate into ongoing or planned trials, without compromising or interrupting their primary trial objectives.

If the right clinical trials are identified and the pilot project framework is well implemented, the preliminary evidence collected would increase our understanding on the feasibility and effectiveness of using ctDNA as a monitoring tool in clinical trials that investigate the efficacy and safety of immune checkpoint inhibitors either used as monotherapy or in combination.

Virtual ctDNA data repository

ctDNA has been and is currently being collected in clinical trials. These rich datasets are currently stored in isolated silos, which preclude powerful and robust analyses that measure the association between plasma response and therapeutic effect. Aggregating these existing datasets in a central virtual repository would allow for datasets to be analyzed together, enabling researchers to draw more significant conclusions and promoting a more refined understanding of plasma response to various therapies, such as chemotherapy, targeted therapies, and immunotherapies.

The working group proposes to explore the creation of a central virtual ctDNA data repository by bringing different stakeholders across academia and industry together to discuss how already-generated data from individual studies could be brought together in a pre-competitive environment. The overarching goal of this initiative would be to discuss how these data could be brought together, what data could be shared across studies, and how these data would be used to derive more insightful conclusions than isolated and smaller studies with limited sample sizes.

A multi-stakeholder virtual data repository offers potential to generate broad learnings in a pre-competitive fashion to facilitate our understanding of ctDNA changes as a measure of drug effect.

Clinical trials use a range of ctDNA analytical approaches and technologies, but most studies have a common core set of data elements and offer means to calculate the *allelic fraction (AF)* of key cancer-associated genes like EGFR, KRAS, and TP53. A combined analysis of existing datasets offers the potential for several learnings:

1) What magnitude of change in AF portends a better response rate, PFS, or overall survival on therapy (e.g., any change, 50% change, 90% change, or 100% change?)
2) How does the relationship between change in AF differ in patients treated with chemotherapy, targeted therapy, or immunotherapy?
3) What minimum baseline "measurable" AF is needed to be able to accurately detect a response in plasma ctDNA?

These learnings will be helpful in furthering our understanding of plasma response and the use of ctDNA in drug development, but a proper framework that will foster collaborations is critical to ensure such a repository is a successful collaborative tool. The working group has put together a list of considerations and questions that begins to explore the potential design and implementation of a virtual data repository that would host ctDNA data to explore plasma response (Table 4).

This type of repository would be beneficial for understanding how best ctDNA could be used in drug development and would help inform future initiatives that seek to operationalize ctDNA in drug development.

Table 4: Considerations for a virtual data repository

Issues	Questions
Core dataset	• What is the minimum core set of data elements that sponsors would feel comfortable sharing as part of a pilot project? • Should raw or analyzed data be uploaded to the repository? • What kind of case report data on clinical response is necessary?
Legal, ethical, and privacy concerns	Are there any legal, ethical, and/or privacy concerns for contributing data to a virtual repository?
Logistical concerns	
Data storage	Where would the data be stored? Would there be a maximum data storage value? Could this data be hosted on a cloud?
Data transfer	How would data be transferred/uploaded?
Blinding	Does the data need to be blinded?
Analytical opportunities	Will the data be analyzed as a meta-analysis, or could the data be combined and analyzed together?

NEXT STEPS

This white paper lays out best practices for ctDNA use in disease monitoring and proposes two collaborative initiatives that could help elucidate how ctDNA may be used in drug development across cancer types and treatment settings. The members of the working group encourage comments and reactions to the best practices and the collaborative initiatives proposed in this white-paper.

Future steps will include the following:

1. ***Friends* will seek to develop a multi-stakeholder consortium: interested members of the academic, diagnostics, government, pharmaceutical, and patient advocacy communities should request to join the ctDNA multi-stakeholder consortium;**
2. **The consortium will meet to discuss the feasibility of the initiatives discussed in this white paper; and**
3. **The consortium will implement the optimal approach to advance our understanding of ctDNA use in drug development**

LIST OF DEFINITIONS

- **Allelic fraction (AF):** refers to the percentage of a sample represented by an allele. Thus, a mutant allele fraction refers to the fraction of alleles (DNA molecules) at a locus that carry a mutation.
- **Cell-free DNA (cfDNA):** total amount of cell-free DNA in plasma or serum, which can be derived from multiple sources, including tumor cells.
- **Circulating tumor DNA (ctDNA):** the fraction of cell-free DNA that originates from tumor cells. The presence of ctDNA in cell-free DNA is generally inferred by the detection of somatic variants, consequently, the presence of ctDNA in cell-free DNA is usually not confirmed until after a ctDNA assay is performed.
- **ctDNA assay:** a clinical test designed to detect somatic variants in cell-free DNA. These encompass a single variant in a gene or broad assays that may interrogate numerous variants in various genes. Other terms to describe ctDNA assays include circulating cell-free plasma DNA assays and plasma genotyping assays.
- **Digital droplet PCR (ddPCR):** a refinement of conventional polymerase chain reaction (PCR) methods where the PCR solution is divided into smaller reactions contained in droplets created through a water oil emulsion technique. Each droplet runs individual PCR reactions independently to directly quantify and clonally amplify nucleic acids in a more accurate and sensitive manner.
- **Liquid biopsy:** a broad category for a minimally invasive test done in a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for fragments of tumor-derived DNA that are in the blood. Tumor genetics or genomics from ctDNA assays are one example.
- **Genotyping (uses for ctDNA):** detection of targetable biomarkers or resistance mutations to guide treatment selection.
- **Monitoring (uses for ctDNA):** repeat assessment to evaluate quantitatively or qualitatively for treatment effect.
- **Cancer detection (uses for ctDNA):** detection of hallmarks of cancer either for initial diagnosis of cancer or for detection of residual cancer at a single high-risk timepoint (e.g. minimal residual disease).
- **Minimum residual disease (MRD):** residual cancer burden persisting in patients considered to be in morphologic remission. Commonly used term in the treatment of blood cancers.
- **Molecular/Plasma response:** changes in ctDNA as a result of a therapeutic intervention.

- **MRD assay:** assay that is tested at some early high impact time to help determine whether a patient is cured or not. Such an assay could also be used at intervals to monitor for recurrence. But the statistical characteristics (and development path, and cost/benefit implications) for a single-timepoint detection assay is quite different than for a multi-timepoint monitoring assay.
- **Next-generation sequencing (NGS):** next-generation sequencing (NGS), also known as high-throughput sequencing, is a term used to describe a number of different modern sequencing technologies that allow us to sequence DNA and RNA much more quickly and cheaply, and as such have revolutionized the study of genomics and molecular biology
- **Real-time PCR (RT-PCR):** real-time polymerase chain reaction (PCR) monitors the amplification of a targeted DNA molecule during the PCR in real-time, and not at its end, as in conventional PCR. RT-PCR can be used quantitatively or semi-quantitatively.
- **Recurrence:** cancer that has recurred usually after a period of time during which the cancer could not be detected. The cancer may come back to the same place as the original (primary) malignancy (local recurrence) or to another place in the body (distant recurrence, or metastasis).
- **Variant allele fraction (VAF):** the fraction of alleles in a specimen that contain the variant, or muta tion.

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