Final Topic Refinement Document

Role of Liquid Biopsy in Detection and Management of Cancer in the Medicare Population

ID: MYOE58

Agency for Healthcare Research and Quality

Technology Assessment Program

Mayo Clinic Evidence-based Practice Center

First Posted on January 22, 2021

Updated July 29, 2021 (Added Appendix B)

Key Questions (KQ)

KQ 1: In adults with increased risk for lung, prostate, breast, ovarian, or colorectal cancer (e.g., BRCA carrier at increased risk for ovarian cancer), can a blood based liquid biopsy (defined for this project as circulating tumor cells [CTCs] and circulating tumor DNA [ctDNA]) be used as a targeted screening test?

KQ1a: What are the pre-analytic factors, analytic validity, and clinical validity of a blood based liquid biopsy?

KQ1b: What is the clinical utility of a blood based liquid biopsy?

KQ 2: In adults suspected to have lung, prostate, ovarian, or colorectal cancer (due to symptoms or signs), can a blood based liquid biopsy (defined for this project as CTCs and ctDNA) be used to establish a diagnosis of lung, prostate, breast, ovarian, or colorectal cancer?

KQ2a: What are the pre-analytic factors, analytic validity and clinical validity of a blood based liquid biopsy?

KQ2b: What is the clinical utility of a blood based liquid biopsy?

KQ 3: In adults with an established diagnosis of lung, prostate, breast, ovarian, or colorectal cancer, can a blood based liquid biopsy (defined for this project as CTCs and ctDNA) direct therapeutic decisions?

KQ3a: What are the pre-analytic factors, analytic validity and clinical validity of a blood based liquid biopsy?

KQ3b: What is the clinical utility of a blood based liquid biopsy?

Draft Analytic Framework

Figure 1 A. Liquid biopsy used in a targeted screening paradigm

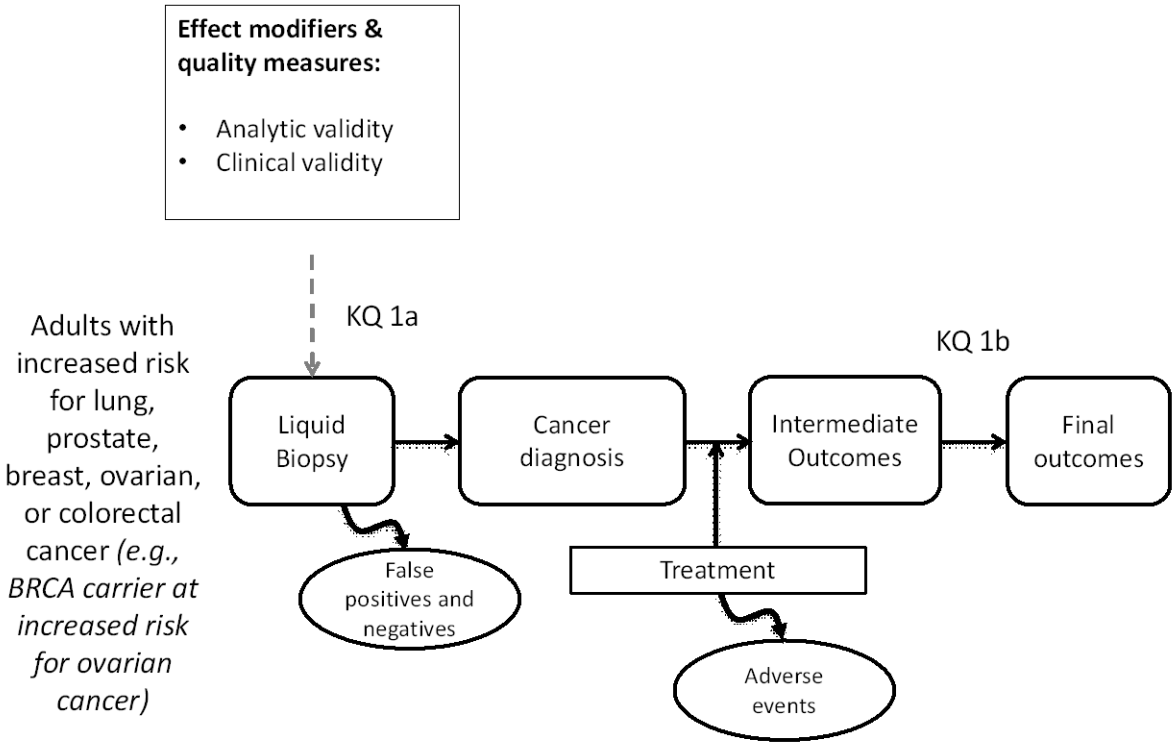


Figure 1 B. Liquid biopsy used in a diagnosis paradigm

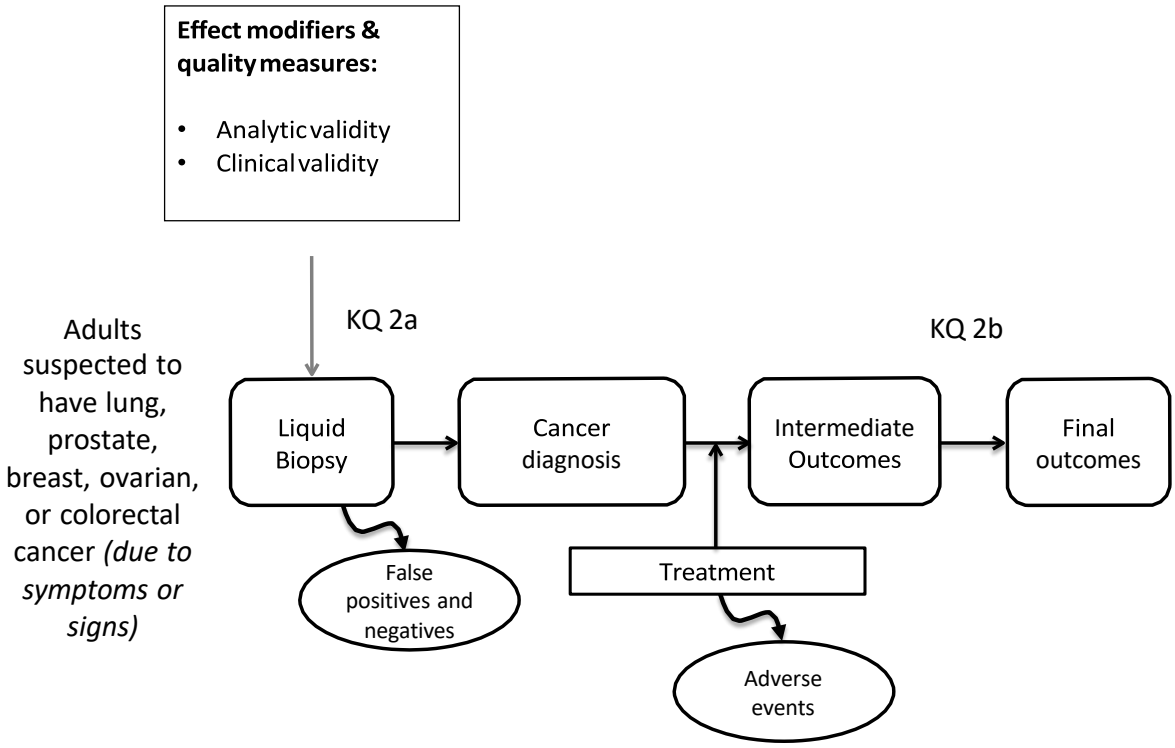
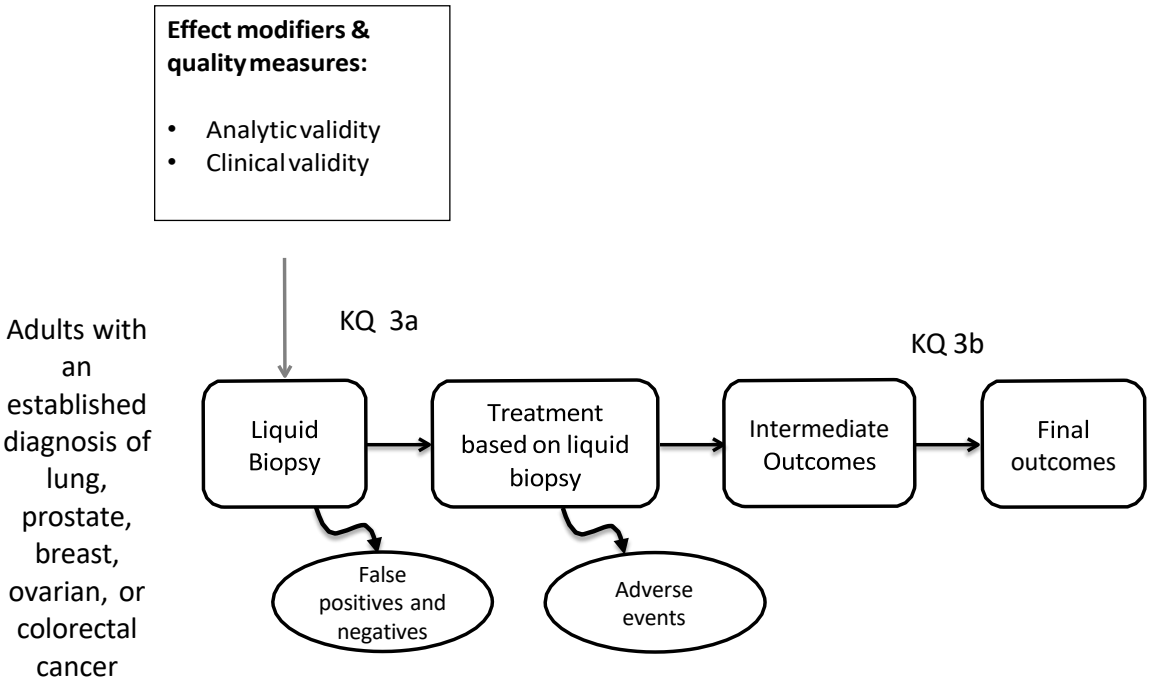


Figure 1 C. Liquid biopsy used in a therapeutic paradigm



Background

Recent technologic advances have allowed for the isolation and analysis of CTCs, ctDNA, and extracellular microvesicles, making the promise of a liquid biopsy possible. A liquid biopsy is defined as the analysis of tumor related material in a sample obtained from the peripheral blood. This material includes intact cells, and nucleic acids (DNA or RNA). Compared to a tissue biopsy, which requires a surgical or image guided procedure to obtain a tissue sample, blood collection by venipuncture is a much less invasive diagnostic approach. Liquid biopsy has been studied in numerous solid tumor types and in hematologic malignancies. However, the majority of the literature in the last decade has been focused on lung cancer, prostate cancer, breast cancer, ovarian cancer, and colorectal cancer. The scope of this topic refinement document focuses on circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) and excludes other biomarkers and methylation based tests.

Regulatory considerations:

Liquid biopsies that are manufactured and performed within a single laboratory [a laboratory with a single certificate issued by the Clinical Laboratory Improvement Amendments (CLIA) program] are considered laboratory developed tests (LDTs). These liquid biopsies are not currently regulated by U.S. Food and Drug Administration (FDA). They are overseen by the CLIA program to ensure analytically accurate and reliable test results. The position of the FDA may change in the future as the FDA has issued a discussion paper on LDTs in 2017 but without an enforceable action.[1] The CLIA final rules specify that the actual performance characteristics of LDTs must be comparable to their claimed specifications in the following areas: accuracy, precision, reportable range of results, reference intervals (normal ranges), analytical sensitivity (detection limit), analytical specificity (interferences/cross reactivity) and any other relevant performance characteristics for the particular test/testing system.

Clinical applications and dilemmas:

Potential clinical scenarios in which a blood based liquid biopsy can be used include screening in asymptomatic individuals at increased risk for cancer, a diagnostic tool in patients suspected to have cancer (such as those with symptoms, thus reducing the need for an invasive tissue biopsy), or as a therapy-guiding tool in patients with established cancer diagnosis (to aid in treatment decisions such as choosing the initial treatment, determining response to a treatment or modifying a treatment).

In terms of screening (identifying disease in an early stage aiming at reducing cancer mortality and morbidity), one important challenge is that screening requires studies with a very large sample size because cancer incidence in asymptomatic individuals is generally low; therefore, sensitivity and specificity cannot be reliably estimated.[2] In addition, because of statutory limitations on screening/prevention services from CMS perspective,[3-6] targeted disease screening would involve a smaller population with a much higher risk, e.g., ovarian cancer in BRCA carriers. In a diagnosis paradigm, a traditional biopsy will likely be preferred in many situations, particularly because patients may anyway require an excision of a mass and a surgical intervention. It remains unclear whether a liquid biopsy can reduce downstream testing, costs and improve hard endpoints, such as survival, when used as a tool to establish diagnosis or guide treatment decisions.

Validity of a medical test:

For a medical test such as the liquid biopsy to be used in practice, several conditions are required.[7] Pre-analytic factors should be evaluated (i.e., the test needs to conform to technical specifications that relate to the collection, handling and storage of the specimen). The test needs to have sufficient analytic validity (i.e., the test needs to measure the substance of interest in an accurate manner concordant with a gold or reference standard), clinical validity (i.e., the test needs to have diagnostic accuracy in classifying the target population) and clinical utility (i.e., the test needs to demonstrate improvement in patients' management and outcomes).

Therefore, a systematic review of the utility of liquid biopsy should collect data on these domains of validity and consider them as markers of methodological quality and possible covariates that can explain heterogeneity.

Current State of the Evidence

We searched six databases (Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations, and Daily, Ovid EMBASE, Ovid Cochrane Central Register of Controlled Trials, Ovid Cochrane Database of Systematic Reviews, and Scopus) from January 1st, 2000 to October 3rd, 2019. We searched comparative studies of liquid biopsy for any type of cancer. We limited our search to randomized controlled trials (RCTs), observational studies, systematic reviews/meta-analyses (SR/MA), and clinical guidelines. No language restriction was used. A total of 8,998 citations were identified, including 8,881 RCTs and observational studies and 117 systematic reviews/guidelines. Then we conduct manual screening of RCT, observational studies, SR/MA, and guidelines. 216 studies were deemed relevant, including 3 RCTs, 198 observational studies, and 12 SR/MA. We present the distribution of studies by cancer, objective (targeted screening in individuals with increased cancer risk/diagnosis and treatment selection/monitoring), and study design in Table 1. In addition, we also identified 128 ongoing trials from ClinicalTrials.gov; which suggests high interest from researchers and manufacturers and indicate that a future systematic review will likely identify more studies than what is included in this topic refinement document.

Table 1: Literature Search and Impact of Scope Decisions on Size of Potential Evidence

Search Description	Objective	Subcategories	Citations
Overall	Targeted screening in individuals with increased cancer risk/diagnosis	TOTAL	160
		RCTs	0
		Observational studies	148
		SR/MA	12
		Guideline	0
	Treatment selection/monitoring	TOTAL	56
		RCT	3
		Observational studies	50
		SR/MA	3
		Guideline	0
Breast Cancer	Targeted screening in individuals with	TOTAL	41

Table 1: Literature Search and Impact of Scope Decisions on Size of Potential Evidence			
Search Description	**Objective**	**Subcategories**	**Citations**
	increased cancer risk/diagnosis	RCT	0
		Observational studies	39
		SR/MA	2
		Guideline	0
	Treatment selection/monitoring	TOTAL	56
		RCT	3
		Observational studies	50
		SR/MA	3
		Guideline	0
Colorectal Cancer	Targeted screening in individuals with increased cancer risk/diagnosis	TOTAL	15
		RCT	0
		Observational studies	14
		SR/MA	1
		Guideline	0
	Treatment selection/monitoring	TOTAL	0
		RCT	0
		Observational studies	0
		SR/MA	0
		Guideline	0
Lung Cancer	Targeted screening in individuals with increased cancer risk/diagnosis	TOTAL	58
		RCT	0
		Observational studies	52
		SR/MA	6
		Guideline	0
	Treatment selection/monitoring	TOTAL	0
		RCT	0
		Observational studies	0
		SR/MA	0
		Guideline	0
Ovarian Cancer	Targeted screening in individuals with increased cancer risk/diagnosis	TOTAL	26
		RCT	0
		Observational studies	24
		SR/MA	2
		Guideline	0
	Treatment selection/monitoring	TOTAL	0
		RCT	0
		Observational studies	0
		SR/MA	0
		Guideline	0
Prostate Cancer	Targeted screening in individuals with increased cancer risk/diagnosis	TOTAL	20
		RCT	0
		Observational studies	19

Table 1: Literature Search and Impact of Scope Decisions on Size of Potential Evidence			
Search Description	**Objective**	**Subcategories**	**Citations**
		SR/MA	1
		Guideline	0
	Treatment selection/monitoring	TOTAL	0
		RCT	0
		Observational studies	0
		SR/MA	0
		Guideline	0

*Ongoing trials identified from ClinicalTrials.gov were not included in this table.

MA = meta-analysis; RCT = randomized controlled trial; SR = systematic review

Draft Population, interventions, comparisons, outcomes, timings and settings (PICOTS)

Population(s)

- Adult patients (18 years and older)
- Patients at increased risk (KQ1), suspected to have (KQ2), or have an established diagnosis (KQ3), of:
    - lung cancer
    - prostate cancer
    - breast cancer
    - ovarian cancer
    - colorectal cancer

Intervention (test)

- Blood based liquid biopsy based on circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA)

Comparators

- Targeted screening, diagnosis or management without liquid biopsy

Outcomes

- Intermediate outcomes
    - Sensitivity, specificity, inter- and intra-laboratory reproducibility (domains of analytic and clinical validity)
    - Downstream testing and procedures
    - Cancer stage at diagnosis
- Final outcomes
    - Overall survival, and harms

Timing

- Any duration of follow-up

Settings

- Any

Subgroup analyses/possible effect modifiers

- Designated as LDT vs. FDA approved test
- Patient characteristics that may interfere with test performance, such as autoimmune disorders

- Different assays
- Adequacy of pre-analytic factor
- Cancer stage
- For KQ3, treatment type (surgery vs. radiotherapy vs. chemotherapy)

Study design

- Randomized controlled trial
- Longitudinal comparative observational studies

Definition of Terms in the context of this topic refinement document

Term	Definition
Liquid biopsy	A minimally invasive test done on 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.[8]
Pre-analytical factors	Issues regarding collection, handling, transport, processing, and storage of a specimen that may affect the subsequent analysis.[8]
Analytic validity	Ability of an assay to detect and measure, with statistical significance, the presence of a substance of interest accurately, reproducibly, and reliably.[8]
Clinical validity	Ability of an assay to divide, with statistical significance, one population into two or more groups on the basis of outcomes.[8]
Clinical utility	Ability to demonstrate, with statistical significance, improvement in the diagnosis, treatment, management, or prevention of cancer, with the use of the assay compared with not using the assay.[8]

DNA = deoxyribonucleic acid

Abbreviation list

Acronym	Definition
AHRQ	Agency for Healthcare Research and Quality
CLIA	Clinical Laboratory Improvement Amendments
CMS	Centers for Medicare & Medicaid Services
CTC	Circulating tumor cell
ctDNA	Circulating tumor DNA
DNA	Deoxyribonucleic acid
EGFR	Epidermal growth factor receptor
EPC	Evidence-based practice center
FDA	Food and Drug Administration
KQ	Key question
LDT	Laboratory developed tests
PICOTS	Population, interventions, comparisons, outcomes, timings and settings
PMA	Premarket approval

Acronym	Definition
RCT	Randomized controlled trial
RNA	Ribonucleic acid
SR	Systematic review

Role of the Key Informants

Key Informants are the end users of research, including patients and caregivers, practicing clinicians, relevant professional and consumer organizations, purchasers of health care, and others with experience in making health care decisions. Within the EPC program, the Key Informant role is to provide input into identifying the Key Questions for research that will inform healthcare decisions. The EPC solicits input from Key Informants when developing questions for systematic review or when identifying high priority research gaps and needed new research. Key Informants are not involved in analyzing the evidence or writing the report and have not reviewed the report, except as given the opportunity to do so through the peer or public review mechanism. Key Informants must disclose any financial conflicts of interest greater than $5,000 and any other relevant business or professional conflicts of interest. Because of their role as end-users, individuals are invited to serve as Key Informants and those who present with potential conflicts may be retained. AHRQ and the EPC work to balance, manage, or mitigate any potential conflicts of interest identified.

Summary of disposition of public comments to Draft Key Questions and supporting material

- The document in general has received a large number of lengthy and detailed comments; which suggests great interest in the topic (see Appendix B).
- A large number of comments were from industry (manufacturers of particular types of liquid biopsy tests). These comments have been carefully considered in the context of potential conflicts of interest. CMS has provided additional comments, which are included in Appendix A.
- Several comments suggested excluding screening from the proposed systematic review citing lack of evidence and the need for large studies to demonstrate an effect. However, screening was of interest to the patient representative and other stakeholders. New literature about screening also appears to be emerging. Therefore, screening will continue to be one of the paradigms of using liquid biopsy to be evaluated in the proposed systematic review.
- Several comments suggested excluding diagnosis from the proposed systematic review citing lack of evidence and the fact that tissue biopsy will always be needed for starting definitive cancer therapies. However, diagnosis was of interest to some of the stakeholders that we have interviewed. Therefore, screening will continue to be one of the paradigms of using liquid biopsy to be evaluated in the proposed systematic review.
- Some comments suggested expanding the number of malignancies studied beyond the proposed 5 (lung, prostate, breast, ovarian and colorectal cancer). From feasibility standpoint, this may make the scope of the review challenging. In addition,

the interviewed key Informants have advised to focus on these 5 tumors because they are the most common and also because the majority of the available literature will be about these 5 types.

- Some liquid biopsy manufacturers recommended expanding the type of assay, for example to ones studying exosomes. Key informant interviews have suggested that these other types are experimental and advised to evaluate assays that measure CTCs and ctDNA.
- Some comments addressed various issues about pre-analytic factors, analytic and clinical validity, and clinical utility. In general, these domains remain critical to evaluate in the proposed systematic review regardless of paradigm (i.e., screening, diagnosis, guiding-therapeutic decisions).

Key Question changes

Public comments were discussed with Centers for Medicare & Medicaid Services (CMS) and Agency for Healthcare Research and Quality (AHRQ) in June 2020 as part of topic refinement. Based on the discussion, these changes were made:

KQ 1: we have focused the question to include patients with increased risk for lung, prostate, breast, ovarian, or colorectal cancer, and added the BRCA carrier as an example to emphasize that this question focuses on targeted screening of high risk individuals.

KQ 2: we clarified that patients suspected to have lung, prostate, breast, ovarian, or colorectal cancer, are patients with symptoms or signs suggestive of cancer who need a diagnostic test (as opposed to a screening paradigm).

All KQs: We added new subgroup analyses by cancer stage and treatment type (surgery vs. chemotherapy vs. radiotherapy). We emphasized the importance of hard endpoints, particularly, overall survival. We used a broad term for the comparators, which is" screening/diagnosis/management without liquid biopsy", in order to include tissue biopsy and other tests, and to include any study settings. We added cancer stage at diagnosis and downstream testing and procedures as intermediate outcomes.

References

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Appendix A: Sponsoring Partner Comments

The EPC received the following comments from CMS:

- CMS notes the relationship between ILLUMINA and GRAIL who each made many comments.
- CMS notes the 9/9/2020 initial public offering by GRAIL and their screening test using methylation.
- CMS notes the role of a Mayo physician as a key informant for this project and as an investigator for a key GRAIL study—a conflict not known by CMS.
- CMS notes the 2019 departure of Josh Ofman (gastroenterologist) from Amgen to GRAIL and ILLUMINA's panel linkage to Amgen's drug panitumumab.
- CMS notes the many comments trying to get screening and diagnosis eliminated from the project and how those commenters wanted assessment of how the test could be used to "help management".
  - No mention was made of the specific pairing with certain drugs.
  - No mention was made of issues (utility) when there was more than one mutation at a single time or when there were serial mutations after evolutionary pressure.

https://www.genomeweb.com/molecular-diagnostics/illumina-receives-fda-approval-companion-dx-run-miseqdx#.X4zFRVhlLIU

Illumina has received US Food and Drug Administration approval for a companion diagnostic test, which it has been developing with Amgen, to run on its MiSeqDx system, the company said after the close of the market on Thursday.

The Extended RAS Panel analyzes 56 variants in the KRAS and NRAS genes to determine whether patients will benefit from Amgen's Vectibix (panitumumab), which is approved for patients with metastatic colorectal cancer who have wild-type KRAS and NRAS genes. Illumina will begin shipping the panel in the third quarter.

Last December the agency approved Foundation Medicine's FoundationFocus CDxBRCA test to identify advanced ovarian cancer patients who have mutations in their BRCA1 and BRCA2 genes and are therefore more likely to benefit from Clovis Oncology's PARP inhibitor Rubraca (rucaparib).

And last week the FDA approved a test developed by Thermo Fisher Scientific in collaboration with AstraZeneca, Pfizer, and Novartis, to identify non-small cell lung cancer patients who are best responders to those pharmaceutical companies' respective drugs.

'The largest application we can imagine': Illumina and Grail CEOs defend their deal to investors ***By*** *Matthew Herper @matthewherper September 25, 2020*

Shares of Illumina, the leader in DNA sequencing, have dropped 13% since news leaked last week that the company would be buying Grail, a startup developing a blood test to detect cancer. Obviously, not every investor loves the $8 billion deal.

But in an interview with STAT, the CEOs of the two firms — Illumina's Francis deSouza and Grail's Hans Bishop — said it would take time for investors to understand the advantages of combining the two firms. They argue that is particularly true in regard to the potential market for Grail's two tests: Galleri, which will aim to detect cancer early in apparently healthy people, and a second, unnamed test, which is being designed to test for potential cases of cancer in patients who have symptoms of the disease.

Filed 9/9/2020
https://www.statnews.com/2020/09/25/illumina-grail-investors-deal/
Grail, a spin out of genome sequencing firm Illumina, filed a preliminary prospectus for a $100 million initial public offering (IPO) last week with the U.S. Securities and Exchange Commission (SEC). This move precedes the company's anticipated 2021 launch of a multi-cancer liquid biopsy screening test for use in asymptomatic individuals over the age of 50.

Grail expects to launch their liquid biopsy product Galleri as a lab-developed test next year. Galleri relies on a targeted methylation sequencing panel to identify more than 50 types of cancer across different stages of disease. Additionally, the blood-based test is designed to help clinicians identify a cancer's tissue of origin. The company is planning for commercialization of their product, and a premarket approval application for a next- generation version of the test has been scheduled for submission in 2023.

Appendix B: Public Comments on Key Questions

Name	Harry B. Burke, MD, PhD
Affiliation	Professor of Medicine Uniformed Services University of the Health Sciences
Key Question 1	KQ1 and KQ2 only differ in terms of the threshold. If there is a high threshold for the diagnosis then the liquid biopsy will, many times, indicate risk of disease. If there is a low threshold for diagnosis then the liquid biopsy will, many times, be diagnostic.
Key Question 2	
Key Question 3	
Analytic Framework	
Background	
Population(s)	
Intervention	
Comparators	
Outcomes	
Timing	
Settings	
Subgroup Analyses	
References	You need to add several references. Tie J, Cohen JD, Wang Y, Christie M, Simons K, Lee M, Wong R, Kosmider S, Ananda S, McKendrick J, Lee B, Cho JH, Faragher I, Jones IT, Ptak J, Schaeffer MJ, Silliman N, Dobbyn L, Li L, Tomasetti C, Papadopoulos N, Kinzler KW, Vogelstein B, Gibbs P. Circulating Tumor DNA Analyses as Markers of Recurrence Risk and Benefit of Adjuvant Therapy for Stage III Colon Cancer. JAMA Oncol. 2019 Oct 17;5(12):1710-7. Burke HB, Kopetz S. Is the Patient Cured? JAMA Oncol. 2019 Oct 17.
Comments	This is a nice start to a very complex subject.
Title	
Duration	

Name	Theo deVos
Affiliation	Epigenomics Inc.
Key Question 1	KQ1: Currently, KQ1 refers to “adults at risk for lung, prostate, breast, ovarian or colorectal cancer.” We suggest that this statement be modified to include the terms asymptomatic and average risk, reflecting a screening test scenario - : “In asymptomatic adults at average or increased risk for lung, prostate, breast, ovarian or colorectal cancer”. KQ1a: This should be reported as unique to each of the five cancers listed. KQ1b: Microsimulation analysis informed by clinical validity data and population data should be considered as an acceptable approach to establish or augment clinical utility.
Key Question 2	
Key Question 3	
Analytic Framework	
Background	General: The scope of this technology assessment proposal is very broad, covering multiple cancers as well as three broad categories of clinical application. The clinical context as well as the value of test performance characteristics (eg. sensitivity & specificity) will vary for each cancer / clinical application combination. For example, in a screening scenario, the importance of the false positive rate may vary greatly for each cancer type, depending on the risks associated with the follow up diagnostic procedure(s). Therefore, it will be essential to assess each cancer and clinical application independently rather than considering generic performance criteria to address all the potential cancer types and varied clinical context. Regulatory considerations: Cancer screening tests have been classified as Class III devices requiring Premarket Approval (PMA) prior to commercial use. This is an important distinction as not all diagnostic or disease management liquid biopsy tests have this level of regulatory stringency. This regulatory classification together with the associated clinical evidence requirements, and the typically low prevalence of disease, requires that screening tests be clinically validated using very large prospective clinical trials. Furthermore, long-term clinical utility outcomes are typically evaluated through microsimulation analysis, as the scale and time-frame required to demonstrate long-term benefits such as life-years gained is often not feasible and cost-prohibitive, particularly for less aggressive and slow progressing cancers. We therefore suggest appropriately scaled clinical validity trials and assessment of clinical utility by microsimulation analysis to be included in the assessment of liquid biopsy screening tests. Clinical Applications and dilemmas: A number of specific technical and study design concerns were raised, which can be addressed through design of the test device, and trial design for validation. -Low abundance of target sequencing; High sensitivity PCR and increased sample volume address this issue, allowing for detection of single genome equivalent of target per mL of plasma. -Large patient studies required low prevalence: For screening tests, a PMA approval should be required with requisite large studies to establish clinical validity, and augmented with published peer-reviewed microsimulation analysis to model utility. -Autoimmune disorders increase cell-free DNA: Highly specific PCR reactions overcome this challenge. -Non-randomized studies leading to bias:

	For screening indications requiring a PMA approval, evidence typically includes prospective collections in the intended use population, with comparison to reference standards. These study designs provide sufficient data for clinical validation.
Population(s)	For screening, the intended use population is typically asymptomatic, average risk and screening eligible as defined by screening guidelines set by either the United States Preventive Services Task Force (USPSTF) or professional societies.
Intervention	
Comparators	For screening, comparators should include “Current Screening Methods and adherence to screening guidelines”.
Outcomes	For screening, outcomes should incorporate microsimulation modeling to establish clinical utility.
Timing	
Settings	For screening, settings should include primary care as well as outreach settings for the medically underserved.
Subgroup Analyses	
References	
Comments	With advances in Nucleic Acid and Multiomic devices that use body fluid samples for non-invasive or minimally invasive testing, we appreciate the efforts AHRQ is undertaking in their Technology Assessment, and the opportunity to provide comments on the assessment plan. These comments are primarily focused on the use of liquid biopsy for cancer screening.
Title	
Duration	

Name	Trisha Brown
Affiliation	Illumina
Key Question 1	In the general comments, I review in more detail why Illumina believes that screening should be out of scope for this review. If screening continues to be included, the review should not be limited to these 5 cancer types, but include all solid tumors. Four of these tumor types listed already have screening technologies that are endorsed by USPSTF guidelines, setting a different standard and way to identify "at risk" adults, whereas other cancer types would require a better definition of "at risk" adults. While comparators are discussed under PICOTs, the comparators for screening a very different from therapy determination and may need to be addressed individually under the key questions. Analytical validity should incorporate sub-bullets to be able to address the questions uniquely for cfDNA vis CTC, and separate out individual technologies. Each potential genetic testing or staining technology has a different sensitivity and specificity that are dependent on the percentage of tumor DNA or cells in the blood, and if the technology is attempting to measure a single or multiple mutations, copy number variants, or gene expression.
Key Question 2	In the general comments, I review in more detail why Illumina believes that diagnosis should be out of scope for this review. If diagnosis continues to be included, the review should not be limited to these cancers, but include all solid tumors. While comparators are discussed under PICOTs, the comparators for "a suspected diagnosis" can vary by tumor type and may need to be addressed individually under the key questions. We also believe that this TA should focus on cfDNA, and separate out CTC for a separate review. If CTC are included, then exosomes should be included as well. Analytical validity should incorporate sub-bullets to be able to address the questions uniquely for cfDNA vs CTC vs exosomes, and separate out individual technologies. Each potential genetic testing or staining technology has a different sensitivity and specificity that are dependent on the percentage of tumor DNA or cells in the blood, and if the technology is attempting to measure a single or multiple mutations, copy number variants, or gene expression.
Key Question 3	It is our opinion this question and TA should not be limited to these five cancer types, but include all solid and hematological tumors as there are established Medicare local coverage determinations (LCDs) whereby Medicare Administrative Contractors (MACs) already cover liquid biopsy-based tumor profiling for all solid tumors. While comparators are discussed under PICOTs, the comparators can vary by tumor type and may need to be addressed individually under the key questions. CTC should be excluded for this review. If included, then exosomes should be included as well. Analytical validity should incorporate sub-bullets to be able to address the questions uniquely for cfDNA vs CTC vs exosomes, and separate out individual technologies. Each potential genetic testing or staining technology has a different sensitivity and specificity that are dependent on the percentage of tumor DNA or cells in the blood, and if the technology is attempting to measure a single or multiple mutations, copy number variants, or gene expression.
Analytic Framework	Figure 1 A: Screening. As stated in the general comments, Illumina believes screening should be excluded from this review. The figure assumes that clinical utility goes as far downstream as outcomes post-treatment. This may be true for some tumor types without screening currently available, but overall treatment efficacy is not the primary purpose of a screening test. The purpose of a screening test is to identify potential cancer in an asymptomatic person. The clinical utility is the ability of the screening test to more accurately identify people most likely to have cancer as early as possible compared to the comparator, with limited related morbidity from false positives. For example, a test that screens positive for cancer would ideally have a high enough sensitivity and specificity to limit the need for unnecessary invasive biopsies or exposure to radiation through imaging. If screening continues to be an aspect of this review,

	then intermediate outcomes such as diagnostic yield, stage at diagnosis, and time to treatment should be emphasized in addition to overall outcomes. Fig. 1 B. As stated in the comments, Illumina believes diagnostic testing should be excluded from this review. Fig. 1 C. Liquid biopsy can be used at different time points post-diagnosis, and should include: choosing initial treatment, monitoring response to treatment, and modifying treatment
Background	Extracellular microvesicles are a legitimate source of material in a liquid biopsy, but are not mentioned beyond the background. We believe the review should focus only on cfDNA, but if CTC are included, then exosomes should also be included for thoroughness.
Population(s)	As defined in the background, liquid biopsy can analyze tumor related cells, DNA, RNA, and proteins in the blood. The types and breadth of analysis possible is significant, and as such, Illumina recommends focusing this review on therapeutic applications, and separate out screening and diagnosis into separate reviews. The comparators for screening or diagnostic applications are often not biopsies, but a wide array of other laboratory tests and imaging. In addition, as the Medicare population already has coverage for pan-cancer liquid biopsies through the MACs, it is recommended that a pan-cancer approach be taken.
Intervention	
Comparators	If screening and diagnosis are included, relevant comparators are missing that include, but are not limited to: - Other laboratory tests such as PSA, hCG, CA125, tumor-based gene expression tests, calcitonin, HE4, NMP-22 etc. - Imaging such as ultrasound, PET scan, X-ray, mammogram, etc. For use in a monitoring capacity (captured within KQ3), relevant comparators would include imaging methods.
Outcomes	For screening applications, “stage at initial diagnosis” should be considered as a relevant intermediate outcome For monitoring applications, “time to detection of recurrence” should be considered as a relevant intermediate outcome For diagnostic and treatment selection applications, “adverse outcomes from invasive biopsy” should be considered a relevant intermediate outcome For treatment selection applications, “actionable biomarker information” (e.g., based on OncoKB rating) should be considered a relevant intermediate outcome. Suggest clarifying what “treatment response recurrence” means Personal utility should be a relevant outcome
Timing	
Settings	If screening and diagnosis are included, these settings are not sufficient. Most screening tests are recommended by a primary care provider, (PCP), and either carried out by the PCP or non-oncology specialist, such as a colonoscopy or mammogram.
Subgroup Analyses	Illumina believes that screening and diagnosis should not be included in this technology assessment. If they are, then the sub-group analyses will need to be considered independently for screening, diagnosis, and management as the issues represented in the sub-group analysis are significantly different between each population.
References	Additional references to consider: - Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA. J Clin Oncol. 2014;32(6):579-586.
Comments	Thank you for undertaking this important topic, and allowing Illumina and the public to provide feedback on the technology assessment framework.

	The scope of clinical use for liquid biopsy is large. While the background accurately describes the potential for screening, diagnosis and management of cancer, the majority of commercially available tests and clinical evidence published to date are focused on the management of cancer using cfDNA. To make this review relevant, timely and streamlined in terms of appropriate comparators, clinical validity, and clinical utility, it is recommended to focus solely on the management of cancer using cell free DNA, and consider screening and diagnosis, and circulating tumor cells, in separate technology assessments in the future. We believe that including screening and diagnosis applications and CTC would be premature at this point in time. Focusing the technology assessment will make the final report more consumable by end users as well. In addition, there are a significant number of tests commercially available and in the pipeline for pan-cancer solid tumor applications, as well as individual cancers such as bladder, renal, and heme. These are important considerations for the Medicare population. Therefore, it is recommended that a pan-cancer approach, more narrowly focused on cancer management and cfDNA, be considered for this technology assessment.
Title	
Duration	

Name	Brian Alexander, MD, MPH
Affiliation	Chief Medical Officer, Foundation Medicine, Inc.
Key Question 1	
Key Question 2	
Key Question 3	In Figures 1A, 1B, and 1C, the liquid biopsy false positives and negatives bubble in the flow diagram doesn't seem to accurately represent the impact of false results. False results would still lead to a cancer diagnosis or treatment based on liquid biopsy results.
Analytic Framework	There is a clinical use case that is missing in the Key Questions; the use of liquid biopsy to determine minimal residual disease in patients that have completed definitive therapy for curable cancer. Foundation Medicine recommends that AHRQ evaluate the validity and utility of a liquid biopsy assay intended to detect residual or recurrent disease.
Background	Regulatory Considerations LDT vs FDA-approved tests: An LDT is not required to be but can be regulated by FDA. Pros and Cons of having tests regulated by FDA: An FDA-cleared assay doesn't need to demonstrate clinical validity, although an FDA-approved assay does. An assay that is not FDA-cleared or approved may still have clinical validity or utility demonstrated. Also, an assay may be FDA-approved for one indication (e.g., detection of EGFR alterations in NSCLC), but may not have analytic validity, clinical validity or clinical utility demonstrated in the indication of interest (e.g., prostate, breast, or CRC). Clinical Applications and Dilemmas Another instance where a liquid biopsy may be preferable over tissue biopsy is when a patient's disease progresses following an initial response to therapy.
Population(s)	
Intervention	
Comparators	The background text mentions that one key reason for use of liquid biopsy is that it might overcome limitations of sampling for tissue biopsy tests, especially in the metastatic setting. However, the stated comparator is tissue biopsy and concordance with a “gold standard” is mentioned in the Validity of a medical test section. This is problematic in that liquid biopsy cannot be both concordant and capable of detecting something another test type missed. There is currently no clear gold standard or reference standard upon which to confirm analytical accuracy for liquid biopsy assays.
Outcomes	For clinical utility, consider additional outcomes related to improvement in patient management. For patients being evaluated in Key Question 3, such measures might be proportion of patients receiving any genotyping, or proportion of patients receiving complete genotyping for all on-label markers, or even proportion of patients receiving complete genotyping in time to initiating therapy.
Timing	
Settings	
Subgroup Analyses	Stage of a patient’s disease is an important factor in liquid biopsy and should be considered as a subgroup analysis.
References	
Comments	On behalf of Foundation Medicine, we appreciate the opportunity to submit comments regarding the Key Question Posting Document and look forward to providing comments on the draft Technology Assessment. Thank you for considering our comments. Please contact me at XXX should you have any questions or if we can provide you with further information.

	Sincerely, Brian Alexander, MD, MPH Chief Medical Officer Foundation Medicine, Inc.
Title	
Duration	

Name	Miguel R. Ossandon
Affiliation	NIH/NCI/Division of Cancer Treatment and Diagnosis/Cancer Diagnosis Program
Key Question 1	
Key Question 2	
Key Question 3	
Analytic Framework	On figure 1B is reed: "Adult at high risk or suspected to have lung, prostate, breast, ovarian, or colorectal cancer". Comment: It is not necessary to include "high risk" in the diagnosis paradigm. If the patient is not suspected to have cancer, but is high risk, then is covered under the screening paradigm.
Background	In the section: "Clinical applications and dilemmas" in the third paragraph is read "In the literature, there are a few instances in which a liquid biopsy was used as a diagnostic tool. For example, testing the peripheral blood to detect stage I to IV colorectal cancer(4)" Comment: 1) Reference 4 is more like an example of liquid biopsy as screening tool. 2) One example of liquid biopsy used as a diagnostic tool, is the evaluation of CTCs on patients with detected lung nodules to rule out malignancy and avoids an unnecessary needle biopsy. Cancer Cytopathol. 2020 Apr 22. Ruth L Katz, Tanweer M Zaidi et al."Identification of Circulating Tumor Cells Using 4-color Fluorescence in Situ Hybridization: Validation of a Noninvasive Aid for Ruling Out Lung Cancer in Patients With Low-Dose Computed Tomography-Detected Lung Nodules.
Population(s)	
Intervention	
Comparators	
Outcomes	
Timing	
Settings	
Subgroup Analyses	
References	
Comments	
Title	
Duration	

Name	
Affiliation	Guardant Health
Key Question 1	
Key Question 2	
Key Question 3	
Analytic Framework	The proposed analytic framework does not take into account rapidly evolving body of research on liquid biopsy. A thorough examination of the literature must acknowledge research conducted outside of the U.S. and reflect the significant advances made since the previous topic refinement in October 2018. As liquid biopsy becomes more widely used, any evaluation of outcomes should not be defined by an outdated reference standard.
Background	There are two areas in which liquid biopsy is poised to radically transform clinical practice in oncology. The first is signaling the presence of an already (molecular) metastatic status in an otherwise phenotypically local disease and the second is forecasting the outcome of systemic therapy in metastatic disease. In both clinical settings, ctDNA acts as synthetic proxy of the tumor biology: in the first case by foreshadowing metastatic spread, signaled by the persistence of minimum residual disease (MRD) after surgery, that distinguishes between indolent and aggressive, metastatic-prone tumors. and in the latter by heralding an inherent resistance to therapy. The utility of liquid biopsies to detect mechanisms of primary and acquired resistance to treatments has been extensively investigated alongside with ctDNA application to direct additional lines of therapies and to guide re-challenge protocols. (Parseghian CM et al. Anti-EGFR-resistant Clones Decay Exponentially After Progression: Implications for anti-EGFR Re-Challenge. Ann Oncol. 2019 Feb 1;30(2):243-249. doi: 10.1093/annonc/mdy509.) ctDNA can be exploited to monitor clonal evolution and identify heterogeneous resistance mechanisms to drug exposure. (Siravegna et al. How Liquid Biopsies can change clinical practice in oncology. Ann Oncol. 30: 1580-1590, 2019) AHRQ should continue to closely monitor the growing body of research being presented on numerous liquid biopsy assays under development before rendering a decision on these emerging use cases.
Population(s)	
Intervention	
Comparators	Tissue biopsy was regarded as the “standard procedure” for molecular detection and was indispensable in decision-making concerning treatment of patients with advanced NSCLC before 2016. However, tumor tissue is often not available due to the invasiveness and the failure of getting enough tumor tissue for further detection of gene variations. Especially for patients whose tumors have developed resistance to targeted-therapy, re-biopsy is extremely difficult due to the suboptimal clinical condition. (Wu, Z et al Update on liquid biopsy in clinical management of non-small cell lung cancer. OncoTargets and Therapy 2019:12, 5098) Liquid biopsy has widely demonstrated to be a viable surrogate for tumor tissue for noninvasive assessment of tumor specific biomarkers and can be potentially used for a variety of clinical and investigational applications. (Santarpia M et al. Liquid biopsy for lung cancer early detection; J Thorac Dis 2018 10 (Suppl 7):S882-S987) It is a source of fresh tumor-derived material, unhampered by preservatives. Sampling the blood is minimally invasive and avoids the complications of biopsies. Recent large studies comparing the performance of ctDNA analysis to tissue biopsy have demonstrated the clinical value of the liquid biopsy approach. (Leighl NB et al. Clinical Utility of Comprehensive Cell-Free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-Small Cell Lung Cancer. Clin Cancer Res 2019; DOI: 10.1158/1078-0432.CCR-19-0624) (Douillard JY et al. Gefitinib treatment in EGFR mutated caucasian NSCLC: circulating-free tumor DNA as a surrogate for determination of EGFR status. J Thorac Oncol 2014;9:1345-53)

	(Mok T, Wu YL, Lee JS, et al. 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. Clin Cancer Res 2015;21:3196-203.) (Nakamura Y. Yoshino T. Clinical Utility of Analyzing Circulating Tumor DNA in Patients With Metastatic Colorectal Cancer. Oncologist. 2018 Nov;23(11):1310-1318. doi: 10.1634/theoncologist.2017-0621. Epub 2018 Apr 26.) In 2018, Aggarwal et al. at the University of Pennsylvania conducted a retrospective analysis of the real-world use of Guardant360 in the care of 323 consecutive metastatic non-small cell lung cancer patients, half of which were tested at the first line. First, the study found that tissue genotyping failed in nearly half (44%) of the 71% of patients in whom the physician elected to use tissue genotyping, which demonstrates how large of a challenge tissue-based genotyping presents in a real-world setting. Secondly, in those patients for whom both tissue and plasma results were available at diagnosis, concordance of the two was very high, at nearly 90%. And thirdly, the addition of Guardant360 nearly doubled the proportion of patients identified with standard of care actionable biomarkers from 21% to 36%, further demonstrating that real-world use of Guardant360 substantially increases the number of treatment opportunities for patients. Finally, and perhaps most importantly, 86% of patients treated based on Guardant360 results achieved either a complete response, partial response or stable disease further demonstrating the high clinical accuracy of Guardant360 testing. These results, combined with patient satisfaction with the relative ease of providing blood rather than a solid tissue sample, suggest a clinical strategy of pursuing plasma NGS first, then tissue NGS if plasma NGS cannot detect relevant mutations. (Aggarwal C et al. Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non-Small Cell Lung Cancer. JAMA Oncology 2019 Feb 1;5(2):173-180. doi: 10.1001/jamaoncol.2018.4305.) There are inherent challenges in using tissue biopsy as the reference standard for liquid biopsy. Many patients have no access to receive tumor biopsy due to the invasiveness of the procedure or the tumor tissue obtained is not sufficient for detection of gene alterations. Moreover, tumor heterogeneity makes the tumor biopsy in one site not able to cover the comprehensive genomic profiles. (Wu, Z et al Update on liquid biopsy in clinical management of non-small cell lung cancer. OncoTargets and Therapy 2019:12, 5098) (Sai-Hong Ignatius Ou. Liquid Biopsy to Identify Actionable Genomic Alterations. American Society of Clinical Oncology Educational Book 38 (May 23, 2018) 978-997. DOI: 10,1200/EDBK_199765.) In many regards, liquid biopsy is proven to produce results that tissue cannot. In evaluating the role and clinical utility of liquid biopsy, we maintain that AHRQ will need to determine the correct comparator - and the most appropriate modeling or statistical technique to apply to ensure that the relative benefits of each test are able to be compared indirectly, the example of plain radiography compared to computed tomography (CT) imaging is a relevant example. Tissue biopsy alone is insufficient. What process has AHRQ used for other emerging technologies to avoid comparing to an outdated standard?
Outcomes	When compared to tissue, liquid biopsy has a significantly shorter turnaround time. A 2018 prospective, multicenter, head-to-head study of SOC tissue-based genomic testing to plasma-based comprehensive cfDNA genomic testing revealed liquid biopsy results via Guardant360 were returned significantly faster than tissue, by almost a week. The median turnaround time for tissue genotyping was 15 days. This long turnaround time is due largely to the practical reality that many newly diagnosed patients require a repeat biopsy to obtain tissue for genotyping as do all acquired resistance patients. (Leighl NB et al. Clinical Utility of Comprehensive Cell-Free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-Small Cell Lung Cancer. Clin Cancer Res 2019; DOI: 10.1158/1078-

	0432.CCR-19-0624) If oncologists can obtain results faster via liquid biopsy, this can increase the patient's likelihood of getting on an effective first-line treatment such as targeted therapy or immunotherapy. (Aggarwal C et al. Clinical Implications of Plasma-Based Genotyping With the Delivery of Personalized Therapy in Metastatic Non-Small Cell Lung Cancer. JAMA Oncology 2019 Feb 1;5(2):173-180. doi: 10.1001/jamaoncol.2018.4305.) A study with a large clinicogenomic database assessed close to 29,000 patients that were treated in 275 oncology practices. Within that dataset there were just over 4,000 patients with NSCLC. The study demonstrated that when patients had a targetable driver mutation and were treated with targeted therapy, they had a median overall survival of 18.6 months vs patients with a targetable mutation that were not treated with targeted therapies at 11.4 months. Roughly 50% of the patients with a targetable mutation were not treated with a targeted therapy, despite the significant increase to overall survival. Length of time to obtain tissue based CGP results may have been a contributing factor. (Singal G Association of patient characteristics and tumor genomics with clinical outcomes among patients with non-small cell lung cancer using a clinicogenomic database. JAMA. 2019;321(14):1391-1399. doi:10.1001/jama.2019.3241) Research shows that relying solely on tissue testing can limit treatment options and result in lack of response to immunotherapy. In the aforementioned NILE study, it concluded that 30 percent of patients with newly-diagnosed NSCLC can be treated successfully with targeted therapies, often yielding higher response rates than chemotherapy or immune checkpoint inhibitors. (Leighl NB et al. Clinical Utility of Comprehensive Cell-Free DNA Analysis to Identify Genomic Biomarkers in Patients with Newly Diagnosed Metastatic Non-Small Cell Lung Cancer. Clin Cancer Res 2019; DOI: 10.1158/1078-0432.CCR-19-0624). If ctDNA is proven useful in these scenarios, treatment-related costs and patient exposure to unnecessary drug-related toxicities can be minimized. (Siravegna et al. How Liquid Biopsies can change clinical practice in oncology. Annals of Oncology 30: 1580-1590, 2019) Any evaluation of the clinical utility should acknowledge the advantage gained in time to treatment from the accelerated TAT associated with liquid biopsy.
Timing	
Settings	
Subgroup Analyses	Designated as LDT vs. FDA approved test. As mentioned previously, oncology care and liquid biopsy are both rapidly evolving fields with new targeted treatments being released quarterly and new biomarkers with FDA approved targeted therapies emerging yearly. In order to keep pace with the evolving field, LDT testing will need to continue to be part of the testing landscape to ensure broad access to care for new and emerging biomarkers. Guardant Health is pursuing FDA approval for a CDx version of Guardant360, however an LDT will continue to be available to ordering physicians with biomarkers that were discovered and placed in the panel after the CDx panel was locked to enable the analytical and clinical validation required for an FDA submission. As new biomarkers are added LDTs will continue to be part of the landscape to ensure that the comprehensive coverage is available for all biomarkers. The current construct of FDA approved tests will most likely make FDA approved tests lag in content by at least 12-18 months. Restricting the landscape to an LDT-only landscape would significantly limit access to care for patients. (Correction, last sentence of original submission) Designated as LDT vs. FDA approved test. Restricting the landscape to an FDA-only landscape would significantly limit access to care for patients.

References	
Comments	Guardant Health is a pioneer in non-invasive cancer diagnostics and comprehensive genomic liquid biopsies. The company's proprietary digital sequencing technology is transforming cancer treatment by providing an accurate and precise picture of the individual genomic alterations that cause tumors to grow, change, and develop resistance to treatment. Guardant Health has combined decades of scientific research, advances in laboratory technology, and breakthrough innovation in liquid biopsy to create a test that has already handled tens of thousands of samples. We appreciate AHRQ's attention to this important topic and welcome the opportunity to provide comments on the Key Question Posting Document for Role of Liquid Biopsy in Detection and Management of Cancer in the Medicare Population. Liquid biopsy research has expanded considerably over the past decade, generating a rapidly growing area of interest in oncology. As AHRQ examines the research landscape for liquid biopsy tests, we hope the agency will acknowledge the range of clinical applications for this technology and solicit stakeholder input - especially from laboratories at the forefront of this research - before advancing policy in this area. The high interest in liquid biopsies is evident in the rapid increase in the use of liquid biopsy in clinical trials, comparative molecular studies, and published studies. (Cescon DW et al. Circulating tumor DNA and liquid biopsy in oncology. Nature Cancer 1, 276-290 (2020) Any topic review of liquid biopsy will need to reflect the pace of innovation in this space; thus, a singular timepoint assessment would not be appropriate because it would be outdated virtually the moment it is published. We would recommend that AHRQ conduct a literature review as frequently as every three years in light of the dynamic nature of liquid biopsy research. The date from which literature is included in reviews is equally relevant, Guardant Health believes that due to the aforementioned technological obsolescence that we see in the field, the current earliest date from which literature should be drawn is 2016.
Title	
Duration	

Name	Zivjena Vucetic, MD PhD
Affiliation	Clinical Genomics, Inc.
Key Question 1	
Key Question 2	
Key Question 3	
Analytic Framework	
Background	
Population(s)	
Intervention	
Comparators	
Outcomes	
Timing	
Settings	
Subgroup Analyses	
References	
Comments	We appreciate the opportunity to submit comments on the proposed Key Questions related to the Role of Liquid Biopsy in Detection and Management of Cancer in the Medicare Population. Clinical Genomics has extensive experience in the field of liquid biopsy for the detection and management of colorectal cancer (CRC) patients. Since 2017, our CLIA-certified and CAP-accredited laboratory in Bridgewater, NJ has provided a circulating tumor DNA (ctDNA) assay for the management of patients previously diagnosed with CRC. This assay is a laboratory-developed test (LDT) and is regulated under federal and state laws and regulations. Additionally, we have developed a ctDNA screening test for CRC and we are in the regulatory process with FDA to obtain Premarket Approval for use of this test in the US market. Both assays detect aberrant methylation in multiple genes that are hypermethylated in CRC tissue and detectable as ctDNA in plasma. During the decade-long process to develop and validate these assays, we evaluated a range of blood-based biomarkers for CRC, including somatic mutations, circulating tumor cells (CTCs), proteins, exosomes, and mRNA transcripts, and we determined that detection of aberrant gene methylation offers the preferred balance of sensitivity, specificity, reproducibility, and cost. We have also learned that the pre-analytic aspects of sample testing have a significant impact on the performance characteristics of an assay. For example, tube selection for blood-based assays and processing to plasma are critical to assay sensitivity and specificity. With respect to the assay itself, extraction of DNA, methods for exposing targeted methylation CpG islands, library preparation, etc. likewise are critical to understand the application and limitations of liquid biopsy methods. As a general comment to all three Key Questions, we believe that AHRQ research should consider how choice of biomarkers in liquid biopsy tests impacts the pre-analytic characteristics, analytic validity, clinical validity and clinical utility for each clinical use (screening, diagnosis, therapy management). The term “liquid biopsy” covers a wide array of technologies that allow for the detection of cellular, genetic and/or epigenetic biomarkers of cancer. Therefore, the Intervention (test) considered should encompass all liquid biopsy biomarkers and technology and not be limited to a single format or methodology, for example,

	mutation analysis, which is the only ctDNA technology mentioned in the background of the public request. For each of the Key Questions, we believe there is a diversity of answers for sub-Key Questions a and b that is driven by the specific solid tumor type that is being considered. Each of these five tumor types in question (lung, prostate, breast, ovarian and colorectal cancer) have different molecular, clinical and demographic characteristics that will impact questions of pre-analytical steps, analytical validity, clinical validity, and clinical utility. It is likely that this research could be broken into five separate projects, each with the same Key Question set, but focusing only on a population comprised of patients at risk, suspected to have, or who have an established diagnosis of one of the solid tumor types. With respect to KQ2, liquid biopsy tests are intended to indicate the presence and/or nature of tumor DNA in circulation. While this information may aid in risk-stratification of patients suspected to have cancer or assessing disease stage, establishing a definitive diagnosis of cancer is usually accomplished via an established clinico-pathological reference method, such as tissue histopathology, radiologic imaging, or visual observation (via endoscopy or surgery). Further, as you consider KQ2b, we encourage you to add an intermediate outcome measure for the rate of curative intent surgery. Extensive literature examining CRC recurrence indicates that median overall survival is significantly influenced by the clinician's ability to attempt curative intent treatment, with those patients who receive curative intent treatment demonstrating dramatically longer median OS. With respect to PICOTS criteria, we suggest the following: - Clinical setting should correspond to clinical specialty regularly involved in caring for the patient at each specific point of cancer care (screening, diagnosis and therapeutic). For example, CRC screening is usually performed in primary care or gastroenterology setting, while oncology setting (outpatient or hospital) is appropriate for therapy and follow up of CRC patients. - Choice of gold standard comparators should be carefully considered for each of the five cancer types and may differ based on the screening, diagnosis or treatment paradigm. Tissue biopsy may be a relevant comparator in the diagnostic paradigm for some cancers, however in the therapeutic paradigm other standard of care comparators should be considered (imaging, clinical, etc.). Additional considerations and questions that may be worthwhile for inclusion in the context of the Key Questions: - Whether a liquid biopsy is likely to be influenced by non-tumor origin cell-free DNA such as from leukocytes or as a consequence of tissue trauma associated with surgery. - Whether a liquid biopsy result is likely to be influenced by chemotherapy or radiotherapy. - Whether a liquid biopsy provides actionable information for the clinical team. Can the test be used to inform drug selection? Does the test identify people who should undergo more intense monitoring or therapy. - For early cancer detection, how specific is the test in relation to tissue of origin, stage of neoplasia and malignant invasion?

	- Does the liquid biopsy identify molecules that are directly or indirectly associated with neoplastic transformation, e.g. is the liquid biomarker released by the tumor or by non-tumor tissue in response to the tumor (e.g. sentinel markers, etc.).
Title	
Duration	

Name	Girish Putcha
Affiliation	Freenome
Key Question 1	KQ 1: 1. It is unclear why only lung, prostate, breast, ovarian and colorectal cancer (CRC) have been chosen, especially given the variability in the recommendations and strength of evidence cited by, for example, the United States Preventive Services Task Force (USPSTF) (1). (Grade A for cervical and CRC; B for breast and lung; C for prostate and D for ovarian) If cancers such as ovarian (for which the USPSTF actively recommends against screening in asymptomatic individuals) are to be included, we suggest revising the scope to include other similar cancers (e.g., pancreatic) since "universal," "pan-", or "multi-" cancer screening using liquid biopsy approaches have been proposed. 2. CTCs and ctDNA do not represent the only biomarkers that may be useful for a blood-based screening test. (For example, other potentially useful biomarkers include (but are not limited to) microRNA, exosomes, and serum or plasma proteins.) We therefore recommend that AHRQ consider focusing on the intended use (i.e., screening in asymptomatic individuals) versus focusing on specific biomarkers or biomarker classes (e.g., ctDNA) when considering questions of clinical validity (CV) and clinical utility. The "pre-analytic" validity is typically characterized as part of the analytic validity (AV), and the evidentiary requirements for AV (and hence pre-analytic validity) will vary significantly based on the specific biomarkers (or types of biomarkers), analytical methods, and intended use. 3. Depending on how one defines "liquid biopsies", the use of such tests for cancer screening is in its infancy, with few such tests currently validated and available for clinical use. Therefore, the peer-reviewed published literature for this intended use of liquid biopsies will be limited, and the proposed technology assessment (TA) may be premature. As discussed below in greater detail (under "General Comments"), we recommend that AHRQ remove KQ1 from the TA. 4. The population identified is "adults at risk for lung, prostate, breast, ovarian, or colorectal cancer." We recommend that AHRQ describe the population as "asymptomatic average-risk adults" and specify the age ranges appropriate for screening since, for example, the USPSTF recommends different ages (and in some cases genders) for screening for the different cancers listed, such as 50-75 for CRC, 55-80 with a history of smoking for lung, etc (1). KQ1a: The "pre-analytic" validity is typically characterized as part of the AV, and the evidentiary requirements for AV (and hence pre-analytic validity) will vary significantly based on the specific biomarkers (or types of biomarkers), analytical methods, and intended use. Similarly, consistent with an almost-unanimous recommendation at the March 9, 2020 FDA public workshop entitled "Detecting Circulating Tumor DNA for Cancer Screening," CV for any screening test for cancer should be performed in a cancer-specific manner based on its intended use using the "gold standard" for diagnosis, which typically requires histopathological confirmation (2). KQ1b: Using current USPSTF recommendations for reference, the clinical utility of screening for certain cancers (e.g., cervical and colorectal) in asymptomatic adults is well-established and accepted, whereas that of others (e.g., prostate) is context-dependent and still others (e.g., ovarian, pancreatic, and thyroid) may have no net benefit or harms that outweigh the benefits (1). Therefore, the evidentiary requirements for the clinical utility of any screening test, liquid biopsy or otherwise, should also vary by cancer type. Specifically, robust demonstration of AV

	and CV should suffice for certain cancers (e.g., cervical and colorectal) but not for others (e.g., ovarian), which will require robust demonstration of clinical utility in addition to AV and CV. In addition, clinical utility requires consideration of not only clinical performance metrics, such as sensitivity and specificity, but also adherence. As stated by William Grady, Professor of Medicine in the Division of Gastroenterology at the Fred Hutchinson Cancer Research Center and a former member of the CRC screening panel for the National Comprehensive Cancer Network (NCCN), "Screening tests that have low compliance rates . . . are not effective clinically even if they have adequate technical performance." For example, in the case of CRC, improved adherence translates directly into decreased mortality and improved quality of life, with each 1% increase in adherence equalling a 1.2-1.5% decrease in CRC-specific mortality (3, 4). Reference(s): 1. https://uspreventiveservicestaskforce.org/uspstf/topic_search_results?topic_status=All&category%5B%5D=15&age_group%5B%5D=10&type%5B%5D=5&searchterm= 2. https://www.fda.gov/media/137482/download 3. Meester RG, Doubeni CA, Zauber AG, et al. Public health impact of achieving 80% colorectal cancer screening rates in the United States by 2018. Cancer. 2015;121(13):2281â€2285. doi:10.1002/cncr.29336 4. Levin TR, Douglas A. Corley DA, Jensen CD. et al., Effects of Organized Colorectal Cancer Screening on Cancer Incidence and Mortality in a Large Community-Based Population. Gastroenterology 2018;155:1383-1391. doi.org/10.1053/j.gastro.2018.07.017
Key Question 2	KQ2: Figure 1b for KQ2 describes the use of liquid biopsies for "diagnosis" in "adults at high risk or suspected to have lung, prostate, breast, ovarian, or colorectal cancer." Currently, diagnosis of these cancers (and solid tumors more generally) requires histology or cytology. We therefore recommend that AHRQ clarify whether "diagnosis" is the correct intended use here. (Note there appears to be a discrepancy between the language in KQ2, which describes the intended use population as "adults suspected to have lung, prostate, breast, ovarian, or colorectal cancer" and Figure 1b, which describes the population as "adults at high risk or suspected to have lung, prostate, breast, ovarian, or colorectal cancer." These should be reconciled for clarity.) KQ2a: As stated above, the "pre-analytic" validity of a test is typically characterized as part of the AV, and the evidentiary requirements for AV (and hence pre-analytic validity) will vary significantly based on the specific biomarkers (or types of biomarkers), analytical methods, and intended use. KQ2b: As stated above in KQ2, the "diagnosis" of solid tumors generally requires histology or cytology, so liquid biopsies are rarely if ever used for this purpose today. More generally, unless there is a truly pathognomonic biomarker, "diagnosis" is rarely established by a single test, liquid biopsy or otherwise, depending instead on a combination of inputs, such as a

	patient's medical history, physical examination, and laboratory test results. We therefore request AHRQ to clarify whether "diagnosis" is the correct intended use for KQ2. A more appropriate application of liquid biopsies may be for surveillance in high-risk populations, but only where such surveillance already has demonstrated clinical utility. As with screening in KQ1, the patient management pathways and clinical utility of surveillance differ for different cancers. For example, the clinical utility of surveillance for CRC in patients diagnosed with hereditary nonpolyposis colorectal cancer (HNPCC) or familial adenomatous polyposis (FAP) is well accepted, but may be less well established for other solid tumors listed (1). Reference(s): 1. National Comprehensive Cancer Network. Genetic/Familial High-Risk Assessment: Colorectal.(Version 3. 2019). https://www.nccn.org/professionals/physician_gls/pdf/genetics_colon.pdf
Key Question 3	KQ3: KQ3 pertains to use of liquid biopsies "to guide therapeutic decisions" in "adults with established diagnosis of lung, prostate, breast, ovarian, or colorectal cancer." We request that AHRQ clarify the specific intended uses under consideration. For example, "guiding therapeutic decisions" could encompass the initial selection of therapy (e.g., targeted or immuno-oncology treatments), minimal residual disease (MRD) monitoring, or recurrence detection, among others. In each case, studies to support the CV and clinical utility of a test will be meaningfully different. (Note that the FDA already has a regulatory framework for companion diagnostics and the use of biomarkers for treatment selection, wherein clinical validity and clinical utility are inextricably linked.) KQ3a: As stated above, the "pre-analytic" validity of a test is typically characterized as part of the AV, and the evidentiary requirements for AV (and hence pre-analytic validity) will vary significantly based on the specific biomarkers (or types of biomarkers), analytical methods, and intended use. KQ3b: The question describes "adults with established diagnosis of lung, prostate, breast, ovarian, or colorectal cancer." As discussed above, context is critical, and the intended use must be clearly and carefully defined. For example, FDA-cleared or approved companion diagnostics currently exist for only the following cancers: acute myelogenous leukemia (AML), B-cell chronic lymphocytic leukemia (CLL), breast, cholangiocarcinoma, chronic myelogenous leukemia (CML), gastric/gastroesophageal, melanoma, myelodysplastic syndrome/myeloproliferative disease (MDS/MPD), non-small cell lung, ovarian, pancreatic, metastatic castrate-resistant prostate, systemic mastocytosis, and urothelial cancer (1). Therefore, FDA-approved companion diagnostic tests for therapy selection have demonstrated clinical utility for these cancers, but it is unclear whether such clinical utility exists for other cancers, including some listed in KQ3. Therefore, in the specific case of biomarkers used for companion diagnostic tests, establishing AV may suffice, but for biomarkers lacking companion diagnostic status, or for cancers for which the clinical utility of a specific biomarker(s) has not been established, demonstration of CV and clinical utility may be required. Similarly, as described below (under "General Comments"), the clinical utility of other intended uses potentially encompassed by

	KQ3 (e.g., MRD or recurrence testing) will vary for different types of cancer, even among the five cancers listed. Reference(s): 1. https://www.fda.gov/medical-devices/vitro-diagnostics/list-cleared-or-approved-companion-diagnostic-devices-vitro-and-imaging-tools
Analytic Framework	As stated above, there appears to be a discrepancy between the language in KQ2, which describes the intended use population as "adults suspected to have lung, prostate, breast, ovarian, or colorectal cancer" and Figure 1b, which describes the same population as "adults at high risk or suspected to have lung, prostate, breast, ovarian, or colorectal cancer." These should be reconciled for clarity.
Background	1. "Liquid biopsies" as a general term need not be limited to blood, but could include any bodily fluid (e.g., CSF, urine, semen and saliva). 2. Materials need not be limited to only "intact cells, nucleic acids (DNA or RNA), and proteins" but could include other components. For example, even if limited to blood, these could include exosomes, platelets, serum or plasma proteins, etc. 3. Not all "in vitro diagnostic liquid biopsy test kits" must undergo "premarket approval." Depending on the intended use, 510(k) or product registration may be appropriate. 4. "Safe and effective" does not necessarily mean "reasonable and necessary," so "clinical utility" is generally a focus for providers (as part of the practice of medicine) and payers, not necessarily regulators, whose focus is typically analytical and clinical validity, not clinical utility. Note also that in the case of a 510(k), the focus is on comparison to a predicate and analytical, not clinical, validity. 5. It is unclear what AHRQ envisions when referring to liquid biopsies used as "a diagnostic tool in patients suspected to have cancer (thus reducing the need for an invasive tissue biopsy)." To our knowledge, there are few, if any, biomarkers in blood, especially for solid tumors, that are truly "diagnostic" or pathognomonic so there may be limited peer-reviewed literature available here. At least today, a diagnosis of cancer typically requires histologic or cytologic evaluation. 6. We suggest AHRQ clarify the following statement ("<1 mutant template molecules per milliliter of plasma, which is beyond the limit of detection of conventional sequencing"). Specifically, is AHRQ referring to "<1 cancer-derived molecule per mL" or "<1 mutation-containing molecule per mL"? This is an important distinction because more recent liquid biopsies may use alternative approaches (e.g., methylation patterns versus mutation detection) to detect cancer-derived molecules. 7. We recommend revising the following statement, which suggests that sensitivity and specificity can never be reliably estimated: "Screening also requires studies with a very large sample size because cancer incidence in asymptomatic individuals is generally low; therefore, sensitivity and specificity cannot be reliably estimated." One possible revision is: "Due to the prevalence of cancer in an asymptomatic average-risk population, sensitivity and specificity require large sample sizes and well-designed studies to be reliably estimated." 8. As indicated above, there are few, if any, liquid biopsies that are used for the "diagnosis" of cancer, especially in solid tumors, so there may be limited peer-reviewed published literature to address this question. The example cited (reference 4) seems to be an abstract that

	describes a test whose intended use is unclear because the patient population for this case-control study is unclear. 9. In addition to the challenge of intra-tumoral heterogeneity, clonal hematopoiesis of indeterminate potential (CHIP) should also be addressed and represents a source of somatic mutations that limit the specificity of ctDNA mutation-based tests. Reference(s): Chabon, J.J., Hamilton, E.G., Kurtz, D.M. et al. Integrating genomic features for non-invasive early lung cancer detection. Nature 580, 245-251 (2020). https://doi.org/10.1038/s41586-020-2140-0
Population(s)	Please see "General Comments," as well as specific comments for KQ1 (comments 1 and 4), KQ1b, KQ2, KQ2b, KQ3, and KQ3b.
Intervention	Please see "General Comments" and Background (comment 2), as well as specific comments for KQ1 (comment 2), KQ1a, KQ2a, and KQ3a
Comparators	Please see "General Comments."
Outcomes	None
Timing	None
Settings	None
Subgroup Analyses	None
References	Reference #4 seems to be an abstract that describes a test whose intended use is unclear because the patient population for this case-control study is unclear.
Comments	On behalf of Freenome, we appreciate the opportunity to submit comments regarding the Key Question Posting Document. At Freenome, our mission is to develop tools to empower everyone to prevent, detect, and treat disease, with an initial focus on colorectal cancer (CRC). Freenome plans to seek FDA approval for a novel artificial intelligence (AI)/machine learning (ML)-enabled multiomics approach to CRC screening that we believe will allow us to achieve greater clinical sensitivity and specificity than conventional blood-based biomarker approaches, particularly for early-stage (I/II) CRC and precancerous polyps. We believe that our test, once approved, will significantly improve current, suboptimal adherence to CRC screening recommendations, which translates directly into decreased patient mortality. Moreover, this test will improve the risk-benefit calculus for patients by increasing the likelihood that those patients directed by a positive Freenome test result to undergo a diagnostic colonoscopy, with its attendant costs and complications, will benefit from it. We therefore have a keen interest in AHRQ's Technology Assessment (TA) and sincerely look forward to working with your organization. To that end, Freenome would like to make the following general comments to supplement the more specific ones above. First, while we and others have presented at various professional society meetings and published in the peer-reviewed literature on the intended uses described in the Key Question Posting Document, liquid biopsies in general represent an emerging technology that are at different stages of clinical development and implementation depending on the specific intended use. Most mature is the use of such tests for therapy selection in late-stage cancer patients, followed by therapeutic monitoring applications (e.g., post-surgical minimal residual disease (MRD) monitoring), followed by screening. We therefore submit to AHRQ that it is premature to do a purely literature-based TA for liquid biopsies for cancer screening, and urge the Agency to remove KQ1. If AHRQ proceeds with the evidence review as drafted, we want to highlight that to our knowledge, AHRQ has not performed such reviews for other more mature

	screening technologies, such as newer stool-based tests, CT colonography, or colon capsule for CRC; 3D breast tomosynthesis or positron emission mammography for breast cancer; the prostate health index (PHI) or 4Kscore for prostate cancer; and so forth. A thorough evidence review of the analytical validity (AV), clinical validity (CV), and clinical utility(CU) of existing screening strategies is an appropriate and necessary precursor to such an analysis focused on a novel and rapidly evolving science (i.e., liquid biopsies), if only to properly contextualize the analysis. Freenome therefore suggests that AHRQ include such an analysis if KQ1 is retained. Second, the scope of the existing proposal is quite significant, potentially representing every intended use for liquid biopsies in cancer. At a minimum, we encourage AHRQ to focus the proposal on the intended uses for which the peer-reviewed published literature is most mature (i.e., therapy selection and, to a lesser extent, therapeutic monitoring), with an understanding that all uses of liquid biopsies are still relatively nascent, and to contextualize even these analyses with a robust assessment of the AV, CV, and CU of alternative approaches, both standard-of-care and emerging. For example, we are not aware whether similar TAs have been performed for tissue-based testing for therapy selection in late-stage cancer patients, or for existing methods for MRD monitoring or recurrence detection. Finally, one size does not fit all in cancer, whether in the context of screening, therapy selection, or therapeutic monitoring. There are over 100 different types of cancer. The evidentiary requirements for AV, CV, and CU for any test, liquid biopsy or otherwise, necessarily and appropriately differ for different cancers because the risks and benefits of subsequent treatments and interventions differ, and therefore so too do the risks and benefits of screening, surveillance, therapy selection, and therapeutic response monitoring. We therefore urge AHRQ, when performing this evidence review, to consider that the evidentiary requirements for AV, CV, and CU necessarily and appropriately vary for different intended uses in different cancers. Thank you for considering our comments. We look forward to the draft Technology Assessment. Please contact me at XXX should you have any questions or seek additional information. Sincerely yours, Girish Putcha, M.D., Ph.D. Chief Medical Officer Clinical Laboratory Director Freenome
Title	
Duration	

Name	Christopher Keir, MD, MS
Affiliation	GRAIL Inc.
Key Question 1	GRAIL believes that based on evolving technological developments and ongoing clinical studies of liquid biopsy for cancer screening that have not yet been published, it would be premature at this time to include this question and KQ2 within the scope of the Technology Assessment. A comprehensive assessment of a field of science to analyze such metrics as pre-analytic, analytic, clinical validity, and clinical utility to help inform important governmental and regulatory decision-making should be conducted at a time when sufficient scientific information and clinical data are available. GRAIL believes that critical data generation to address and guide the Draft Key Questions is still ongoing. In addition, data supporting the measures of clinical utility of a cancer screening application are distinct and not easily combined with a post-cancer diagnosis application.
Key Question 2	See comments to Question 1.
Key Question 3	
Analytic Framework	
Background	
Population(s)	GRAIL believes it is premature to include liquid biopsy for cancer screening within the scope of the Technology Assessment, and urges AHRQ to remove “patients at risk” and “patients suspected to have” from the analysis population.
Intervention	Circulating tumor cells and circulating tumor DNA are purified from blood and analyzed using very different technical pipelines; the presence of CTCs in the blood also denotes a different pathophysiology than ctDNA. Assessment of the clinical validity and
Comparators	
Outcomes	Any outcome measure should be parsed by and based on the technology’s intended use, which further highlights the importance that a technology assessment of using liquid biopsy in a post-cancer diagnosis setting should be separate and distinct from a technology assessment of liquid biopsy in a cancer screening application.
Timing	
Settings	The setting is dependent on the developer’s intended use for the technology (e.g., screening applications would generally be in a primary care setting). Thus, it is important that a technology assessment of using liquid biopsy in a post-cancer diagnosis
Subgroup Analyses	
References	Seminal papers on liquid biopsy usage for cancer tracking and treatment response A;, Diaz LA; “Liquid Biopsies: Genotyping Circulating Tumor DNA.” Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, U.S. National Library of Medicine Bettegowda C; “Detection of Circulating Tumor DNA in Early- And Late-Stage Human Malignancies.” Science Translational Medicine, U.S. National Library of Medicine Chen G; “Clinical, Molecular, and Immune Analysis of Dabrafenib-Trametinib Combination Treatment for BRAF Inhibitor-Refractory Metastatic Melanoma: A Phase 2 Clinical Trial.” JAMA Oncology, U.S. National Library of Medicine Dawson SJ;”Analysis of Circulating Tumor DNA to Monitor Metastatic Breast Cancer.” The New England Journal of Medicine, U.S. National Library of Medicine Diaz LA; “The Molecular Evolution of Acquired Resistance to Targeted EGFR Blockade in Colorectal Cancers.” Nature, U.S. National Library of Medicine, Diehl F; “Circulating Mutant DNA to Assess Tumor Dynamics.” Nature Medicine, U.S. National Library of Medicine

	Hamakawa T; “Monitoring Gastric Cancer Progression With Circulating Tumour DNA.” British Journal of Cancer, U.S. National Library of Medicine Jenkins S; “Plasma CtDNA Analysis for Detection of the EGFR T790M Mutation in Patients With Advanced Non-Small Cell Lung Cancer.” Journal of Thoracic Oncology : Official Publication of the International Association for the Study of Lung Cancer, U.S. National Library of Medicine Lipson EJ; “Circulating Tumor DNA Analysis as a Real-Time Method for Monitoring Tumor Burden in Melanoma Patients Undergoing Treatment With Immune Checkpoint Blockade.” Journal for Immunotherapy of Cancer, U.S. National Library of Medicine, M;, Rossi G; “Promises and Pitfalls of Using Liquid Biopsy for Precision Medicine.” Cancer Research, U.S. National Library of Medicine, Merker JD; “Circulating Tumor DNA Analysis in Patients With Cancer: American Society of Clinical Oncology and College of American Pathologists Joint Review.” Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, U.S. National Library of Medicine, Mok T; “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.” Clinical Cancer Research : an Official Journal of the American Association for Cancer Research, U.S. National Library of Medicine, Reinert T; “Analysis of Circulating Tumour DNA to Monitor Disease Burden Following Colorectal Cancer Surgery.” Gut, U.S. National Library of Medicine, Schmiegel W; “Blood-Based Detection of RAS Mutations to Guide Anti-EGFR Therapy in Colorectal Cancer Patients: Concordance of Results From Circulating Tumor DNA and Tissue-Based RAS Testing.” Molecular Oncology, U.S. National Library of Medicine Tie J; “Circulating Tumor DNA as an Early Marker of Therapeutic Response in Patients With Metastatic Colorectal Cancer.” Annals of Oncology : Official Journal of the European Society for Medical Oncology, U.S. National Library of Medicine, Vidal J; “Plasma CtDNA RAS Mutation Analysis for the Diagnosis and Treatment Monitoring of Metastatic Colorectal Cancer Patients.” Annals of Oncology : Official Journal of the European Society for Medical Oncology, U.S. National Library of Medicine Seminal papers on liquid biopsy usage for cancer screening Chabon JJ; “Integrating Genomic Features for Non-Invasive Early Lung Cancer Detection.” Nature, U.S. National Library of Medicine Wolpin, Brian M; “Performance of a Blood-Based Test for the Detection of Multiple Cancer Types.” Journal of Clinical Oncology, vol. 38, no. 4_suppl, 2020, pp. 283-283. Lennon AM; "Feasibility of Blood Testing Combined With PET-CT to Screen for Cancer and Guide Intervention." Science (New York, N.Y.).
Comments	Thank you for the opportunity to provide responses regarding the Key Question Posting Document. GRAIL, Inc. (GRAIL) is a healthcare company whose mission is to detect cancer early, when it can be treated or cured. GRAIL is focused on alleviating the global burden of cancer by developing pioneering technology to detect and identify more than 50 cancers. We are encouraged by the rapid progress in the field of liquid biopsies. GRAIL continues to publish data that suggests these advancements can change how we screen for and diagnose cancer, which could support earlier treatment. While our progress is promising and holds great potential, the active but ongoing status of more comprehensive research makes it premature at this time for AHRQ to assess liquid biopsy for cancer screening in the proposed Technology Assessment. We believe an AHRQ technology assessment should be guided by the patient population, clinical condition, and clinical use of technology under examination. The current AHRQ proposed scope of work includes very different patient populations in very different clinical settings. In addition, tests used to assess the molecular characterization of a tumor in a cancer patient or to diagnose cancer in an individual with a clear radiologic or other finding, is a very

	different intervention than a test to detect a cancer signal in a normal or high risk individual without known cancer. Moreover, this scope of work is considering a broad range of different technological and scientific approaches to the evaluation of circulating cfDNA and CTCs that make general statements about analytical approaches or validation less than useful, as these novel technologies are demanding new approaches to these assessments. For example, the analytical validation approach to single analyte mutation panels will by their very nature be quite different from the analytical validation approach for "pattern recognition" tests based on a constellation of patterns using machine learning algorithms. These methodologies are currently under development and under active exploration by scientific experts and regulators. As a brief summary, key studies on ctDNA usage provide the preclinical motivation for clinical trials on the validity and utility of liquid biopsy for applications of cancer progression, treatment selection and response, and cancer evolution (Diaz Jr, Hamakawa, Bettegowda, Diehl, Reinert, Lipson, Tie). Use of liquid biopsy to detect EGFR mutations in NSCLC has already been approved in the USA; in Europe, liquid biopsies for EGFR mutations in NSCLC or KRAS mutations in CRC are also approved (Jenkins, Schmiegel, Vidal). Proof of concept studies have already shown that ctDNA titer can be used clinically to measure tumor response to therapy in lung, colorectal, and breast cancer (Mok, Tie, Dawson, Chen), and numerous additional clinical studies are currently underway to demonstrate clinical validity and utility for cancer management (Rossi). Recently, academic institutions and companies have begun to investigate the use of ctDNA (or liquid biopsies, including circulating tumor proteins, RNAs, epigenetic materials, and microvesicles) for early cancer detection purposes, with the goal of refining the sensitivity and specificity of liquid biopsy technology to be usable for diagnostic applications (Chabon, Wolpin, Lennon). Compared to the use of liquid biopsy in patients with established cancer diagnoses outlined above, the use of liquid biopsy for early detection or screening purposes requires significant clinical evidence to illustrate the difference in study populations with the goal of detecting any possible cancers detectable by liquid biopsy technology rather than following the progression of one known cancer. In screening populations, the pretest probability of any given cancer is low, so liquid biopsy performance characteristics must have very high specificity to prevent overdiagnosis and false positives and should be coupled with robust sensitivity. Additionally, the ability to predict tissue of origin is a crucial component for both the clinician and patient. Technology for use of liquid biopsy as a screening tool is rapidly under development and continuing to evolve as evidenced by three recent informative publications: Diehn laboratory at Stanford (Chabon), GRAIL (Wolpin), and CancerSEEK (Lennon). These technologies are showing significant promise (Merker) and we anticipate the results of prospective studies will become available in the near future to demonstrate the clinical utility of a liquid biopsy for cancer screening. We are concerned that addressing these diverse populations, clinical conditions, and broadly different technological approaches in a single technology assessment will create enormous confusion in the field and potentially take away from the value of an informative AHRQ review of specific technology in a well-defined clinical area such as diagnosed cancer. As a result of the ongoing research to establish the performance and health outcomes of liquid biopsy for cancer screening, we believe it would be premature at this time for AHRQ to conduct a comprehensive technology assessment that would inform and shape governmental and regulatory decision-making. We respectfully submit these comments with the intention of ensuring that AHRQ is able to perform an informative review that can influence the clinical practice of medicine. We think once validation and utility studies for screening and diagnosis tests have been conducted and published, AHRQ should evaluate this topic in the specific patient populations in which such tests have been studied.
Title	
Duration	

Name	Hannah Murphy
Affiliation	Coalition for 21st Century Medicine
Key Question 1	As outlined below (under "General Comments"), the Coalition for 21st Century Medicine (C21) believes it is premature at this time to include liquid biopsy for cancer screening within the scope of the Technology Assessment, and urges AHRQ to revise the key questions to remove this question. In addition, data supporting the analytical validity, clinical validity and clinical utility of circulating tumor cells versus circulating tumor DNA are distinct and not easily combined under a single analysis.
Key Question 2	Data supporting the analytical validity, clinical validity and clinical utility of circulating tumor cells versus circulating tumor DNA are distinct and not easily combined under a single analysis. Also, this assessment should focus on the use of liquid biopsy in patients with established cancer diagnoses at this time because its use in the diagnostics space is still under substantial development and is undergoing rapid change.
Key Question 3	Data supporting the analytical validity, clinical validity and clinical utility of circulating tumor cells versus circulating tumor DNA are distinct and not easily combined under a single analysis.
Analytic Framework	As outlined below (under "General Comments"), the Coalition for 21st Century Medicine (C21) believes it is premature at this time to include liquid biopsy for cancer screening or to establish a diagnosis within the scope of the Technology Assessment, and urges AHRQ to revise the key questions to remove these questions. In addition, data supporting the analytical validity, clinical validity and clinical utility of circulating tumor cells versus circulating tumor DNA are distinct and not easily combined under a single analysis.
Background	
Population(s)	The Coalition for 21st Century Medicine (C21) believes it is premature at this time to include liquid biopsy for cancer screening or for patients suspected to have cancer within the scope of the Technology Assessment, and urges AHRQ to remove the "patients at risk" and the "suspected to have" population from this analysis.
Intervention	Data supporting the analytical validity, clinical validity and clinical utility of circulating tumor cells versus circulating tumor DNA are distinct and not easily combined under a single analysis.
Comparators	Comparators should include a reference standard (tissue biopsy may or may not be a reference standard) AND/OR a standard of care.
Outcomes	
Timing	
Settings	
Subgroup Analyses	
References	
Comments	On behalf of the Coalition for 21st Century Medicine (C21), we appreciate the opportunity to submit comments regarding the Key Question Posting Document. C21 comprises many of the world's most innovative diagnostic technology companies, clinical laboratories, physicians, venture capital companies, and patient advocacy groups. Member companies are developing and offering novel "liquid biopsy" tests that may be used to screen patients at risk for cancer, establish a diagnosis in patients suspected of cancer, and guide therapeutic decision-making in patients with an established cancer diagnosis. Given the Coalition's mission to facilitate development and commercialization of innovative diagnostics to inform important patient management decisions, we have a keen interest in the agency's Technology Assessment on this dynamic and rapidly evolving field. While C21 member companies are developing and performing liquid biopsy assays for all of the use cases described in the Key Question Posting Document, these comments focus on AHRQ's consideration of liquid biopsy as a screening test for adults at risk of certain cancers or suspected to have certain cancers.

	New blood-based molecular diagnostic technologies are emerging that have the potential to address challenges in early detection of cancer (e.g., lack of guideline-recommended screening tests for many types of cancer). These assays, which may allow physicians to detect cancer when outcomes are better and costs are lower, are increasingly supported by foundational evidence establishing their performance. Indeed, member companies regularly present data regarding the performance of these assays at professional society meetings (e.g., American Society for Clinical Oncology (ASCO) and American Association for Cancer Research (AACR)), and this research is increasingly published in peer-reviewed journals. C21 expects there to be a substantial increase in the amount and quality of this data - including published, peer-reviewed data - in the coming years, but at the moment there is not a body of published data on clinical utility to review yet. However, for now, these assays are still in clinical development. As a result, C21 believes it is premature at this time to include liquid biopsy for cancer screening or for use in patients suspected to have cancer within the scope of the Technology Assessment, and urges AHRQ to revise the key questions to remove these questions. Our recommendation is to focus the Technology Assessment on areas where there is a more robust and established evidence base with extensive published, peer reviewed clinical data, multiple tests in the market, and a defined patient population, such as those with existing cancer. In addition, data supporting the measures of clinical utility of a screening application are not easily combined with a post-cancer diagnosis application. C21 thanks AHRQ for the opportunity to comment on the Key Question Posting Document, and looks forward to providing comments on the draft Technology Assessment. Thank you for considering our comments. Please contact me at XXX should you have any questions or if we can provide you with further information. Sincerely, Hannah Murphy Executive Director The Coalition for 21st Century Medicine
Title	
Duration	

Name	Dr. Paul Billings
Affiliation	Natera
Key Question 1	
Key Question 2	
Key Question 3	
Analytic Framework	
Background	
Population(s)	
Intervention	
Comparators	
Outcomes	
Timing	
Settings	
Subgroup Analyses	
References	
Comments	Response to AHRQ Request for Feedback: Key Questions Posting Document for Role of Liquid Biopsy in Detection and Management of Cancer in the Medicare Population To whom it may concern, Natera is the nation's leading provider of molecular diagnostics with products in the fields of Women's Health, Oncology and Organ Health/Transplantation. Utilizing innovations in sample handling, multiplex polymerase chain reaction (PCR) methods and novel bioinformatics, Natera has addressed unmet medical needs by enhancing relevant information delivered by simple blood sampling. We believe that the important work on "liquid biopsy" that AHRQ is considering can be aided by Natera's analysis and experience. Biopsies are a cornerstone of traditional pathological analysis of disease. They are used to confirm the presence of disease, the exact type of disease, and in some cases the extent of disease. In addition, the results of a biopsy can aid in appropriate treatment selection and directing towards long term management guidelines. But tissue biopsies are often invasive, can have associated complications and morbidities, can be open to varying expert interpretations and methodological variances, and may therefore have limited availability when crucial management disease management decisions are made. Recognizing these limitations, attempts have been made to enhance the information deliverable from more accessible sources (saliva, stool, urine, sweat and blood for instance). The term and concept of "liquid biopsy" was formulated over 15 years ago to represent a relatively non-invasive method, using simple small volume phlebotomy, to gain most, all or even supplemental data that would normally be delivered by a tissue sample. The technique has evolved since its initial formulation, and the quality and extent of the data generated have increased substantially. It is therefore crucial that this AHRQ project clearly define the scope of its consideration of "liquid biopsy".

	For instance, tissue biopsy is not generally used as a disease screening tool. But blood sampling may prove to have utility for the purpose of identifying individuals at average or increased risk for a condition. Similarly, tissue biopsy is not a tool for quantifying the extent of disease; miminal/molecular residual disease assessment and quantification, as well as responsiveness to intervention, can be assessed by blood based sampling and the measurement of circulating tumor DNA (ctDNA). Blood is an ideal sample to detect both germline and somatic DNA variations that can be informative about disease origin, diagnosis, prognosis or susceptibility to treatment and is far simpler, faster and less expensive at getting this information than older tissue biopsy approaches. For some use cases, the proper comparator for the use of blood (liquid biopsy) as an disease assessment aid is the traditional information gleaned from proper tissue biopsy analysis. In other situations, tissue is not the "gold standard". For instance, in the assessment of MRD using ctDNA, the proper comparator is clinical assessment with imaging supplementation. Both the sensitivity of detection and quantification of tumor burden have been improved with the newer "liquid biopsy" ctDNA based method. Similarly, standard criteria for evaluating treatment response are enshrined in RECIST 1.1 that uses imaging, not tumor biopsy, as the data source. Furthermore, imaging-based disease assessment is itself a surrogate for clinical outcomes, as progression-free survival is considered a surrogate for overall survival in many clinical trials. But other studies have suggested that imaging is not always well correlated with clinical outcomes, due to false positive and false negative interpretations associated with confounding clinical variables (example??) or well-characterized phenomena like image detected, tumor pseudo-progression. In summary, as the AHRQ clarifies the scope of this potential study, it should understand the value historically of traditional biopsy, what ways newer methods of sampling have supplemented or added to this body of knowledge, and unique applications of blood based methods that have been applied in clinical research and patient care. Natera, with its growing expertise in the use of blood analysis as an aid to patient care, stands ready to assist the agency in completing its work. Sincerely, Paul Billings Chief Medical Officer Natera
Title	
Duration	