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Potential clinical utility of ultrasensitive circulating tumor DNA detection with CAPP-Seq

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Abstract

Tumors continually shed DNA into the circulation, where it can be non-invasively accessed. The ability to accurately detect circulating tumor DNA (ctDNA) could significantly impact the management of patients with nearly every cancer type. Quantitation of ctDNA could allow objective response assessment, detection of minimal residual disease, and non-invasive tumor genotyping. The latter application overcomes the barriers currently limiting repeated tumor tissue sampling during therapy. Recent technical advancements have improved upon the sensitivity, specificity, and feasibility of ctDNA detection and promise to enable innovative clinical applications. In this review, we focus on the potential clinical utility of ctDNA analysis using CAPP-Seq (CAncer Personalized Profiling by deep Sequencing), a novel next-generation sequencing-based approach for ultrasensitive ctDNA detection. Applications of CAPP-Seq for the personalization of cancer detection and therapy are discussed.

INTRODUCTION

We are in the midst of a revolution in molecular oncology that is allowing for increasingly personalized management of cancer patients. The individualization of cancer care will rely on the development of effective targeted therapeutics as well as biomarkers for selecting the appropriate treatments and evaluating their effectiveness. To aid with complex decision making in clinics, improved tools are needed in order to accurately measure disease burden, assess prognosis, and predict response to targeted therapies.

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Circulating tumor DNA (ctDNA) has emerged as a promising cancer biomarker because it provides non-invasive access to cancer DNA. Distinct from circulating tumor cells (CTCs), ctDNA is cell-free and can be collected from peripheral blood plasma, urine, or other bodily fluids. Although more comprehensive, head-to-head comparisons across a larger number of tumor types are needed, several recent studies have suggested that ctDNA may be detectable by deep sequencing-based approaches in a greater proportion of patients than CTCs[1–4]. A major technical challenge in analysis of ctDNA is that the vast majority of the cell-free DNA found in plasma originates from a patient's healthy cells. Therefore, highly sensitive techniques are necessary for reliable detection and quantitation of the tumor-derived fraction. For example, in patients with stage IV non-small cell lung cancer, the percent of circulating DNA that is tumor-derived has been shown to vary between median values of ~0.1% to 5% and is affected by factors such as disease burden and treatment status[5, 6].

Early efforts at detecting ctDNA mostly focused on application of allele-specific real-time quantitative PCR assays[7]. These assays, which utilized technologies such as TaqMan, PNA clamps, and Scorpion Amplification Refractory Mutation System (ARMS), were limited in their applicability to patients with high tumor burden due to their analytical sensitivity and specificity. However, within the past decade several methods have been developed that allow for ultrasensitive detection of ctDNA. These methods have detection thresholds between 0.01% and 0.1% for mutant allele abundance and fall into two main categories – digital PCR (dPCR)[8, 9] and next-generation sequencing (NGS)[5, 10, 11]. The dPCR-based methods have very high analytical sensitivity for minor alleles (~0.01%) with improved specificity and reproducibility as compared with real-time quantitative PCR[12] but generally can only interrogate one or a few genomic positions simultaneously. Additionally, assays must be optimized for each mutation of interest, which complicates clinical implementation.

NGS-based methods for ctDNA detection can simultaneously detect multiple somatic alterations simultaneously. While early NGS-based ctDNA detection platforms had insufficient sensitivity for most clinical applications (>1%)[13, 14], several groups, including ours, have recently developed NGS-based methods that permit ultrasensitive ctDNA detection[5, 10, 11]. Two of these utilized deep sequencing of a limited number of amplicons targeting commonly mutated cancer genes[1, 2, 11, 15, 16]. Although low detection thresholds are achievable with such methods, technical limitations related to multiplexing of PCR assays have to date limited the number of genomic positions that can be interrogated. This complicates potential clinical application since a given small combination of amplicons will not identify a mutation in the majority of patients with most cancers. Moreover, amplicon-based methods are not able to detect most rearrangements and translocations if the exact breakpoints are not known *a priori*.

To overcome these issues, we developed a capture-based NGS ctDNA detection method called CAPP-Seq (CAncer Personalized Profiling by deep Sequencing), which is applicable "off the shelf" to the vast majority of patients with a given cancer type and which can detect all major classes of mutations including single nucleotide variants, indels, rearrangements, and copy number alterations[5]. Capture-based NGS methods enrich for genomic regions prior to sequencing by hybridization of target regions to antisense oligonucleotides. Such methods are scalable such that large portions of the genome can be examined. As a result, CAPP-Seq

can usually identify multiple mutations in any given patient's tumor, which increases its sensitivity and facilitates assessment of intratumoral heterogeneity. These properties make CAPP-Seq an effective tool with which to investigate the potential clinical utility of ctDNA analysis in a variety of contexts.

MEASUREMENT OF DISEASE BURDEN

For a patient diagnosed with cancer, precise measurements of the total body disease burden may have prognostic significance and may be useful for assessing treatment response. Currently, the workhorse for such measurements is medical imaging, including CT, PET, and MRI. Medical imaging consumes up to 6% of the total cost of cancer care in the United States[17], and both CT and PET expose patients to ionizing radiation. Furthermore, response assessment on scans is subjective, imaging has suboptimal resolution for identifying small tumor deposits (< ~1 cm diameter), and it can often be difficult to distinguish local treatment effects from recurrent cancer[18]. Despite these limitations, the use of high-cost medical imaging studies has been on the rise among cancer patients[17].

Quantitation of ctDNA by CAPP-Seq could potentially overcome many of the shortcomings of imaging for measurement of disease burden. Multiple studies have demonstrated that changes in ctDNA levels can reflect treatment response in patients with advanced disease[1, 2, 5, 8, 11, 14, 15]. CAPP-Seq is designed to limit sequencing costs by targeting recurrently mutated genomic regions; current reagents and sequencing costs are approximately $200–$400 per assay, and costs will continue to decrease as NGS technologies mature. Still, there are a number of caveats to consider regarding the potential utility of monitoring disease burden using ctDNA. First, it is not known whether ctDNA is released at the same rate from primary, nodal, and distant metastatic sites. Some variation is likely to be present, based on differences in both tumor cell biology as well as access to the circulation[10]. For example, the blood-brain barrier may limit the passage of ctDNA from the central nervous system into the peripheral circulation[10]. Second, tumor histology likely impacts ctDNA release in ways that are not yet completely understood. Third, although there exists promising data suggesting that ctDNA analysis will be more sensitive than medical imaging[5, 8], this will need to be explored in much larger patient cohorts. Fourth, ctDNA analysis by itself cannot reveal where tumor deposits are located within the body. We therefore envision that ctDNA analysis will be complimentary to standard imaging for disease monitoring.

PROGNOSTIC INDICATOR

There is hope that ctDNA levels could provide added prognostic information beyond standard clinical indices. The correlation between ctDNA levels and traditional stage groupings is imperfect[10]; rather, it appears that total tumor volume better predicts ctDNA levels[5]. Tumor volume measurements derived from medical imaging are frequently found to be strongly prognostic[19, 20], but in patients with metastatic disease precise measurements of tumor volume can be challenging. For these patients, quantitation of ctDNA could potentially be used to identify individuals with worse long-term survival[2, 8].

One particularly exciting application of ctDNA analysis was illustrated by Diehl and colleagues[8]. In their report, the absence of detectable ctDNA following surgery for advanced colorectal cancer identified individuals that remained disease-free for extended periods[8]. In the context of early stage malignancies, detection of minimal residual disease (MRD) post-surgery using ctDNA analysis could distinguish between patients with micrometastases who may derive a significant benefit from aggressive adjuvant systemic therapy and patients without residual disease who could be spared the toxicity of such treatments. For example, the use of adjuvant chemotherapy is controversial in patients with stage I lung cancer or stage II colon cancer because prospective randomized trials have failed to show a survival advantage in unselected populations[21, 22]. By incorporating CAPP-Seq into future clinical trials, patient selection could potentially be optimized when testing adjuvant therapies.

The notion that detection of MRD following treatment can affect prognosis and aid clinical decision-making is not new. MRD analysis is a vital component of post-treatment monitoring in hematologic malignancies and can identify individuals at high risk for relapse despite otherwise displaying complete response to therapy. In this context, MRD analysis involves PCR or multiparameter flow cytometry on cellular material from bone marrow biopsies or peripheral blood. We envision that CAPP-Seq will extend the applications of MRD analysis to solid malignancies for which no similar tests currently exist. CAPP-Seq can detect disease burden below the resolution of medical imaging[5], demonstrating its potential utility in MRD monitoring. While secreted protein biomarkers can serve this function in a subset of patients with a few cancer types, poor specificity limits their utility in many instances. In contrast, patient-specific genetic markers detected by CAPP-Seq are by nature specific to the tumor of interest. Future studies will compare the clinical utility of CAPP-Seq to other available biomarkers for MRD monitoring.

NON-INVASIVE GENOTYPING AND DETECTION OF RESISTANCE MUTATIONS

In the age of personalized medicine, an ever-increasing number of targeted cancer therapies are available to specifically kill tumor cells with defined genetic aberrations. Thus, accurate tumor genotyping has become an essential component of optimal patient selection for these treatments. Unfortunately, there are often practical barriers to adequate tumor tissue acquisition, including risk from invasive procedures, inadequate sample retrieval through needle biopsies, and difficulties of performing repeated invasive procedures over the course of therapy. Non-invasive access to tumor DNA could therefore enable more frequent and reliable tumor genotyping without the risks and discomfort that accompany biopsies. A growing number of companies are now offering or developing ctDNA-based tests in order to address the demand for such analyses.

Currently only a handful of cancer mutations are important for therapeutic decisions. However this list will continue to grow as more targeted cancer therapies are developed and as the mechanisms of resistance to these agents are elucidated. As a capture-based NGS method, CAPP-Seq has the capability to interrogate thousands of genomic loci in parallel for the purpose of non-invasive genotyping. This differentiates it from other methods such as

dPCR or amplicon-based NGS, which have limited abilities to simultaneously interrogate multiple mutations and thus require splitting of a blood sample into separate aliquots. Such subdividing of blood samples is problematic since given the low concentrations of ctDNA that are present in most patients, a particular mutation will only be represented by a handful of molecules in a blood sample. Furthermore, CAPP-Seq has the advantage that in addition to point mutations, it can detect indels, rearrangements and copy number changes, which are also important determinants of response to certain targeted agents[23, 24].

Analysis of ctDNA also offers a strategy for monitoring evolving tumor heterogeneity over the course of therapy, since it simultaneously integrates contributions from cells within a primary tumor as well as from different tumor deposits throughout the body. This is particularly relevant in regards to the emergence of mutations that confer resistance to targeted therapies, which can be readily detected using CAPP-Seq[5]. Ultimately, early detection of such mutations could facilitate modification of therapy at a time when the burden of resistant cells is still low.

CANCER SCREENING

The application of ctDNA analysis that could have the largest impact on patient survival is cancer screening. Many cancers are curable when detected early in their development, and screening programs that identify early-stage tumors have demonstrated important survival benefits[25, 26]. However, screening programs produce large numbers of false positive results, which can cause significant stress and lead to unnecessary invasive procedures[27, 28], possibly degrading survival gains while adding costs to health care systems.

Detection of ctDNA could potentially improve upon the diagnostic accuracy of screening tests by reducing false positive results. However, ctDNA analysis in this context is complicated by the facts that (1) tumors are small and therefore ctDNA concentrations are very low, (2) the specific mutations present in a given patient's tumor are not known, and (3) somatic mutations within circulating DNA may also be present as a result of mosaicism or benign/precancerous lesions[29, 30]. Due to its high analytical sensitivity and specificity as well as ability to simultaneously interrogate thousands of possible mutations, CAPP-Seq could overcome some of these obstacles. In exploratory analyses, we found that CAPP-Seq can be tuned to have a high positive predictive value for lung cancer detection without prior knowledge of tumor genotype. We expect ongoing technological improvements to enable even greater gains in diagnostic accuracy, which ultimately may make ctDNA-based cancer screening feasible. Much like existing screening tests, such ctDNA-based screening would need to be applied to high risk populations in order to limit the impact of false positives and may be best used in conjunction with medical imaging to limit the number of false positive results from both modalities.

CONCLUSIONS AND FUTURE DIRECTIONS

Over the past few years, considerable enthusiasm has developed for the clinical implementation of ctDNA detection technologies. Because ctDNA reflects the genomic changes that occur within cancer cells, these technologies provide non-invasive access to

biomarkers for diagnosis, prognosis, and treatment response assessment. With CAPP-Seq, the possible clinical applications of ctDNA analysis continue to expand, and additional innovations can be expected in the near future. Once thought to be applicable primarily in advanced stage cancers, NGS analysis of ctDNA is now technically feasible in early stages as well. As with every new biomarker, the clinical utility of ctDNA analysis will need to be proven through well-designed clinical trials. However, based on the large amounts of promising data published in this field over the past few years, we anticipate that ctDNA analysis will revolutionize detection and management of cancer in the near future.

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