Editorial

Circulating tumor DNA in relapse prediction: a gold mine on the rise

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Nominated in the top 10 breakthrough technologies in 2015 (1), the use of liquid biopsy in cancer diagnostics has drawn a lot of attention in scientific and industrial communities. It is non-invasive by nature, and can be accessed from a blood draw, from cerebrospinal fluid (2) or even from urine (3). Circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) are among the most promising detection markers in liquid biopsy (4). Although FDA has approved one CTC test kit for prognosis of cancer patients' survival assessment, CTC analysis for clinical implications remains controversial. In contrast, the potential of ctDNA in tumor detection is much more encouraging. Studies demonstrated that the half-life of ctDNA is around 2 hours, allowing the real-time tracking of genomic alternations happening in the tumor (5). Most importantly, detection of ctDNA in liquid biopsy is a promising biomarker for tumor burden, therefore possesses enormous value in early detection of cancer, monitoring treatment response, quantifying minimal residual disease (MDR) and prediction of relapse.

A recent article published by Abbosh *et al.* in Nature joined the list of exciting reports of using ctDNA in MDR monitoring and relapse prediction (6). As part of the umbrella clinical study of TRACERx (7), Abbosh and colleagues tracked 100 patients with early stage non-small cell lung cancer (NCSLC). Lung cancer is the second most common cancer in both men and women and account for 14% of new diagnoses worldwide (8,9). About 85% of lung cancers are classified as NCSLC, which is extremely lethal if advanced or metastatic, with a 5-year survival rate of 1% for stage IV NSCLC (10). Notably, the benefit of adjuvant chemotherapy for NSCLC is as low as 5% (10). Development of a better predictive biomarker for NCSLC relapse and response to adjuvant chemotherapy is therefore in a great demand. In the paper, Abbosh and colleagues investigated the utility of sequencing ctDNA to track evolutionary dynamics and predictability of Postoperative relapse of NCSLC. Patients recruited in the cohort were diagnosed with stage I to III NSCLC. After surgery, bulk tumor biopsies were genomically profiled, and personalized single nucleotide variants (SNVs) panels were designed according to each patient's tumor. By utilizing a platform of multiplexed-PCR followed by NGS, the authors were able to identify up to 30 tumor-specific SNVs for each patient, by sampling patients' plasma ctDNA. The platform achieves a high sensitivity of above 99% for SNV detection at SNV frequency above 0.1% and the specificity of detecting a single SNV was 99.6%. The threshold of ctDNA positive call is present of at least two SNVs.

Empowered by presence of both pathological tumor biopsy and plasma ctDNA, the authors observed a linear relationship between tumor volume and plasma variant allele frequency (VAF) (both log transformed), which is consistent with previous reports on NSCLC and thus enables quantitative estimation of tumor burden (11). A plasma VAF of 0.1% corresponds to 326 million tumor cells. It would be interesting to examine how generalizable

this estimation is for different cancers, because it can be directly compared to the resolution of CT scan, which is the conventional method to detect onset of relapse.

One of the main goals of this study is to examine the capability of detecting MRD and the tumor subclones that drive relapse using patient-personalized ctDNA. To this end, the authors collected pre- and post-operative ctDNA for a sub-group of 24 patients, and patients were followed-up for every 3 to 6 months and up to 31 months for relapse. Of the 14 patients that were confirmed with relapse, 13 of whom had ctDNA with at least 2 SNVs detected, and the median gap between ctDNA detection and relapse confirmation is 70 days. On the other hand, 9 out 10 patients who are ctDNA negative lived disease free within the follow-up time window. The remaining one patient that had ctDNA detected prior to adjuvant chemotherapy, but remained ctDNA negative after the treatment, and was free of relapse 688 days post-surgery. Despite the relatively small sample size, the results are very promising: ctDNA appears to be a reliable biomarker foreseeing postoperative relapse of NSCLC, with both sensitivity and specificity above 90%. Moreover, phylogenic characterization of ctDNA matched clonal SNV to the tumor biopsy in 94% of cases, and 68% for subclonal SNVs. Most impressively, the successive detections of ctDNA matched the clonal structure of the relapsed, metastatic tumor biopsy, after a retrospective examination of post-mortem tumor sample using custom ctDNA array, which further enlightens the power of using ctDNA for MRD detection and therapy guidance.

Although promising, it is import to point out that the study conducted by Abbosh *et al.*, as well as other recent reports on predicting relapse by ctDNA analysis are all based on small scale studies for a specific cancer type (12). To achieve reliable clinical implication, large-scale randomized clinical intervention studies are required to fully evaluate the potential caveats of using ctDNA as biomarkers, and to demonstrate which types of cancer will be specifically benefited from this novel technique. We should also keep in mind that the physiological mechanism of ctDNA release for tumor cells still remains largely unknown. Further investigation unveiling this mystery would ease the interpretation of ctDNA analysis result. However, the most challenging part of making ctDNA-based diagnosis practical is how to enhance the sensitivity of the assay while keeping the cost affordable. In Abbosh *et al.*'s study, the authors estimated a cost of $1,750 cost per patient for sequencing a single tumor region, with five liquid biopsy samples of custom-designed target panel, given a detection limit of 0.01% VAF. Then the questions is that is it good enough. A study on a cohort of 231 colon cancer patients published last year prompts a strong argument on that (13). By using the similar platform, high specificity of 97% was achieved in predicting postoperative relapse in 36 months; however, the reported sensitivity was relatively low. A single plasma sample only yield 48% sensitivity, while the number could boost to 79% when sequencing all samples collected. Apparently, scale-up the number of samplings for ctDNA detection is beneficial, but the companion cost would be increase dramatically too. On the other hand, personally designed mutation panel targeting a small number of genes could still be a rate-limiting step. Alternatively, low pass whole genome sequencing, focusing both SNVs and copy number alterations (CNA) is another heated area that actively researched in the field (14,15). The rationale for using genome-wide signals for ctDNA detection is that, due to the fragmented nature of ctDNA, each locus may offer an independent sampling of the total ctDNA in the body. This offers the possibility to achieve lower limits of detection than thought to be possible based on the number of haploid genome equivalents of ctDNA in a single tube of blood. Moreover, the nucleosome positioning of genome ctDNA could be used to infer tumor lineage (16), which could be critical for early cancer diagnosis. Each of the above methodological improvement would bring in significant benefits toward ctDNA analysis for clinical implementation.

Another key finding of Abbosh *et al.* is that ctDNA detected 94% of clonal SNVs seen in the primary tumor and also foresee the driving SNVs in the metastatic tumors, again demonstrating ctDNA as the real-time, dynamic tracker of the underlying tumor burden. If implemented in large-scale clinical studies, ctDNA profiling can become a high-throughput data generation platform of real-time patient data, where genomic features can be directly linked to clinical outcome. As a result, patient response can be readily monitored, which in turn is the perfect guide for patient stratification in clinical trials. Patients with no improvement in ctDNA test can avoid unnecessary treatment, while patients benefited from the on-trial therapeutics can be further studied by genomic analysis of the time-series of ctDNA data.

Although mostly proof-of-concept, the development of the liquid biopsy field has seen promising successes. The existing challenges and hurdles would be overcome by the discovery of novel biomarkers, as well as the development of more sensitive and accurate ctDNA detecting approaches. With the contribution of scientists, clinicians and the

patients, we are confident that liquid biopsy will continue to transform the dogma of cancer diagnosis and therapeutics which in turn would benefit a lot of cancer patients in the near future.

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