18 NEW METHOD OF ASSESSING TUMOR HETEROGENEITY UTILIZING BOTH CIRCULATING TUMOR DNA AND TISSUE DNA TO PREDICT THE RESPONSE TO IMMUNOTHERAPY

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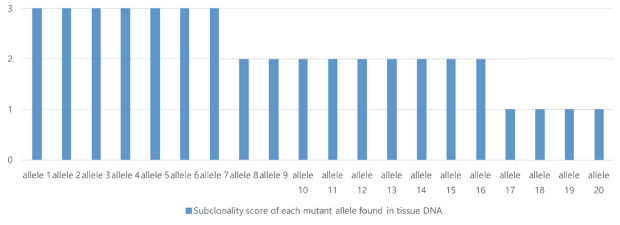
Background Tumor heterogeneity assessment may help predict response to immunotherapy. In melanoma mouse models, tumor heterogeneity impaired immune response.[1] In addition, among lung cancer patients receiving immunotherapy, the high clonal neoantigen group had favorable survival and outcomes.[2] Ideal methods of quantifying tumor heterogeneity are multiple biopsies or autopsy. However, these are not feasible in routine clinical practice. Circulating tumor DNA (ctDNA) is emerging as an alternative. Here, we reviewed the current state of tumor heterogeneity quantification from ctDNA. Furthermore, we propose a new tumor heterogeneity index(THI) based on our own scoring system, utilizing both ctDNA and tissue DNA.

Methods Systematic literature search on Pubmed was conducted up to August 18, 2020. A scoring system and THI were theoretically derived.

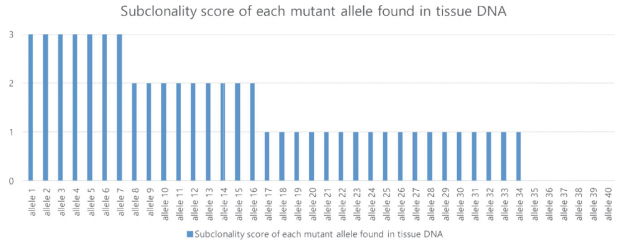
Results Two studies suggested their own methods of assessing tumor heterogeneity. One suggested clustering mutations with Pyclone,[3] and the other suggested using the ratio of allele frequency (AF) to the maximum somatic allele frequency (MSAF).[4] According to the former, the mutations in the highest cellular prevalence cluster can be defined as clonal mutations. According to the latter, the mutations with AF/MSAF<10% can be defined as subclonal mutations. To date, there have been no studies on utilizing both ctDNA and tissue DNA simultaneously to quantify tumor heterogeneity. We hypothesize that a mutation found in only one of either ctDNA or tissue DNA has a higher chance of being subclonal. We suggest a scoring system based on the previously mentioned methods to estimate the probability for a mutant allele to be subclonal. Adding up the points that correspond to the conditions results in a subclonality score (table 1). In a given ctDNA, the number of alleles with a subclonality score greater than or equal to 2 divided by the total number of alleles is defined as blood THI (bTHI) (figure 1). We can repeat the same calculation in a given tissue DNA for tissue THI (tTHI) (figure 2). Finally, we define composite THI (cTHI) as the mean of bTHI and tTHI.

Abstract 18 Table 1 Subclonality score

Condition	point
Not included in the highest frequency cluster by Pyclone	1
AF/MSAF <10%	1
Present in only one of either ctDNA or tissue DNA	1



Abstract 18 Figure 1 Hypothetical distribution of all alleles found in ctDNA bTHI = the number of alleles with a subclonality score greater than or equal to 2/the total number of alleles found in ctDNA = 10/20 =50%



Abstract 18 Figure 2 Hypothetical distribution of all alleles found in tissue DNA tTHI= the number of alleles with a subclonality score greater than or equal to 2/the total number of alleles found in tissue DNA = 16/40 = 40% cTHI= (bTHI + tTHI)/2 = 45%

Conclusions Tumor heterogeneity is becoming an important biomarker for predicting response to immunotherapy. Because autopsy and multiple biopsies are not feasible, utilizing both ctDNA and tissue DNA is the most comprehensive and practical approach. Therefore, we propose cTHI, for the first time, as a quantification measure of tumor heterogeneity.

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0018

19 WHOLE-EXOME SEQUENCING BASED IMMUNOGENOMIC PROFILING WITH POTENTIAL CLINICAL APPLICABILITY IN CIRCULATING CELL-FREE DNA AND TISSUE FROM ADVANCED STAGE COLORECTAL CANCER PATIENTS

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Background Assessing cfDNA at a whole-exome scale (WES) enables comprehensive immunogenomic profiling and interrogation of tumor heterogeneity. We comprehensively investigate genomic alterations and neoantigens in cfDNA at WES-scale using Personalis' NeXT Liquid Biopsy™. Genomic alterations, neoantigens, and molecular tumor micro-environment (mTME) from matched solid tumor are evaluated using Personalis' ImmunoID NeXT Platform®.

Methods Matched plasma, tumor, and adjacent normal tissues were collected from 13 late-stage, treatment-naive CRC patients. cfDNA was extracted and assessed exome-wide, then the mutational landscape and immunogenomic profile were analyzed.[1] gDNA extracted from tumor was analyzed by the ImmunoID NeXT Platform, where somatic variants and

neoantigens were evaluated. RNA analysis of the solid tumor enabled the investigation of the mTME.[2 3]

Results The average number of somatic SNVs in plasma samples was 100.5 (Range 50–250). KRAS, APC, PIK3CA, SMAD4, FBXW7, ARID1A were identified. Specifically, two components of SWI/SNF complex, ARID1A and BRD9, were both mutated in plasma samples, suggesting the potential dysregulation of epigenetic pathways. RTK-RAS and Notch pathways were also frequently mutated. Further, 1,195 somatic events were found in genes not covered by commercially available targeted panels. 27 of these SNVs are in immuno-oncology related genes, which highlight the importance of somatic evidence observable through an exome-scale cfDNA approach. In solid tumor, the average number of detected somatic SNVs was 133.4 (Range 69–230), with similar mutation landscape. Concordance is observed between tumor and plasma samples (mean: 40.6%; range: 15.13%-94.2%). However, a number of variants are plasma-specific, suggesting that cfDNA WES detects tumor mutations that might be missed by a single site biopsy. We evaluated neoantigens and determined that the fraction of variants predicted as neoantigens are similar between plasma and tumor. Importantly, several of the top neoepitopes are uniquely predicted in plasma, suggesting the potential clinical value of using WES cfDNA. RNA-sequencing of solid tumor samples enabled mTME profiling. CD8 T cell immune infiltration, TCR beta clonality and clone counts were low, suggesting these patients have cold tumors. Myeloid dendritic cells and macrophages demonstrated uniform abundance across samples, while B and T regulatory cells showed variable tumor infiltration

Conclusions Results demonstrate potential clinical utility and highlight the advantages of whole-exome scale profiling of plasma and matched tumor samples, which enables a systematic interrogation of tumor biology, including mTME. Notably, a whole-exome based liquid biopsy assay offers indispensable insights that might be otherwise missed by a single site tumor biopsy or targeted liquid biopsy panels.

Ethics Approval The study protocol was in accordance with the tenets of the Declaration of Helsinki. Commercial samples used in this study were procured from Bioreclamation IVT and BioChain following protocols approved by the local Institutional Review Board (IRB) committee. Informed consent forms were obtained from all the human subjects in this study.

Consent N/A

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0019

20 QUANTIFICATION OF SBCMA IN HUMAN PLASMA USING A HIGH-THROUGHPUT MASS SPECTROMETRY WORKFLOW FOR EXPLORATORY, CAP/CLIA OR REGULATED STUDIES

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Background Bioanalytically validated methods are needed for reliable measurement of B cell maturation antigen (BCMA) in clinical samples. BCMA contributes to multiple myeloma (MM) pathophysiology and is targeted by various novel immunotherapies. Soluble BCMA (sBCMA) is a promising novel biomarker for disease monitoring and prediction. To address the need for sBCMA measurement, we developed a mass spectrometry-based assays for the quantitation of total sBCMA in plasma.

Methods Immunoaffinity enrichment of sBCMA from human plasma was performed on a Agilent AssayMap Bravo platform using streptavidin cartridges. Recovered sBCMA protein was digested to peptides using trypsin, spiked with a fixed level of stable isotope labeled (SIL) peptide standard, and analyzed by multiple-reaction-monitoring (MRM) mass spectrometry. The MRM assay targeted a BCMA-specific endogenous peptide used as a surrogate measure of the protein, as well as the corresponding spiked SIL peptide at known concentration. An 8-point external calibration curve was prepared by spiking varying amounts of recombinant BCMA and fixed amounts of SIL peptides in surrogate matrix. Endogenous sBCMA levels were determined by back-calculating against the curve. To assess the performance of the method, $\geq$ 2 precision and accuracy runs were performed. To assess the impact of ligand or drug binding on the developed assay, performance of the assay was tested using plasma spiked at a range of concentrations with rhAPRIL, rhBAFF or simulated mAb drug.

Results The developed assay allowed the quantitation of sBCMA from 1 to 1000 ng/mL. The precision and accuracy at different QC levels was within 20% CV and 20% bias. The presence of binding proteins (rhAPRIL, rhBAFF, simulated mAb) did not interfere with the measurement of sBCMA indicating that the assay measures total sBCMA.

Conclusions We have developed an assay for the absolute quantitation of total sBCMA in human plasma. The assay can be analytically validated and deployed for clinical studies with a $\sim$ 3-day turnaround time.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0020

21 PLASMA-BASED PROTEOMIC PROFILING AS A TOOL FOR PREDICTING RESPONSE TO IMMUNOTHERAPY IN MELANOMA PATIENTS

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Background In recent years, studies have indicated that in response to almost any type of anti-cancer therapy, the patient (the host) may generate pro-tumorigenic and pro-metastatic effects. This phenomenon, called host-response, counteracts the anti-tumor activity of the treatment. We have previously shown that machine learning-based plasma proteomic analysis of the host response may serve as a predictive tool for