

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

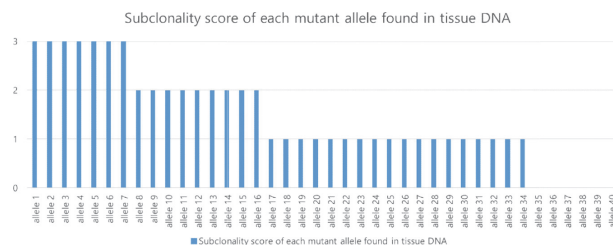
¹Jaeyoun Choi*, ¹Myungwoo Nam, ¹Stanislav Fridland, ¹Jinyoung Hwang, ²Chan Mi Jung, ²Christmann Low, ¹Young Kwang Chae. ¹Northwestern University Feinberg School of Medicine, Chicago, IL, USA; ²Northwestern University Weinberg School of Arts and Sciences, Chicago, IL, USA

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

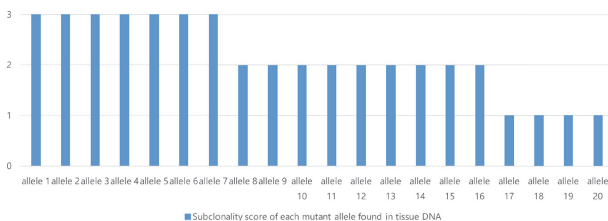
¹Zheng Feng*, ²Juergen Scheuenpflug, ³Mengyao Tan, ⁴Danyi Wang. ¹EMD Serono, A Business of Merck KGaA, Darmstadt, Germany; ²Merck KGaA, Darmstadt, Germany, Darmstadt, Germany; ³Personalis, Inc, Menlo Park, CA, USA; ⁴EMD Serono, Billerica, MA, USA

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

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%