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PP01.62
The Journal of Liquid Biopsy 5 (2024) e1–e32 100240
Non-invasive immunogram to characterize and monitor immune status in non-small cell lung cancer patients treated with immunotherapy.

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Introduction: Non-Small Cell Lung Cancer (NSCLC) represents 80% of lung cancer cases, being one of the most frequent and death causing cancers. Recently developed treatments with immunotherapy have improved patient prognosis. However, a significant number of patients do not respond to treatment, thus there is an urge for biomarkers.
The main objective of this study was to obtain a combination of non-invasive biomarkers capable of predicting which NSCLC patients benefit from immunotherapy.
Methods: This study included 52 advanced-stage NSCLC patients treated with Anti-PD1 or Anti-PD1 in combination with chemotherapy (Anti-PD1 + CT) in the first-line setting. Non-invasive biomarkers were analysed using peripheral blood samples, which were obtained before first cycle and at first response assessment. The potential biomarkers analysed in this study were i) haematological and immunological parameters, ii) immune-related gene expression analysed on Peripheral Blood Mononuclear Cells (PBMCs), iii) T Cell Receptor (TCR-β) repertoire, and iv) HLA genotype. RStudio was used to create multivariate models by applying machine-learning techniques to prioritize the analysed variables with greater influence on patient outcomes.
Results: The integration of the analysed variables has resulted in a proposal of a multivariate model capable of predicting patients with improved outcomes to treatment with anti-PD1 therapy. Our immunogram combined PD-L1 tumour tissue expression with other biomarkers such as Neutrophil-to-Lymphocyte Ratio (NLR) and certain HLA alleles to predict prognosis, in the subgroup of patients treated with anti-PD1 monotherapy.
Furthermore, we developed two independent non-invasive immunograms with the ratios of analytical variables to identify patients with DCB, which could be of significant utility to monitor patients throughout treatment with anti-PD1 or anti-PD + CT. Interestingly, patients with improved prognosis had an increase in the number of TCR-β clones, but also an increase in clonality and higher number of shared clonotypes between pre- and on-treatment samples. Despite immunotherapy-based treatment boosts anti-tumour immune responses, pre-existing anti-tumour immunity might be required to trigger and maintain effective responses.
Conclusion: Altogether, this study highlights the role of composite non-invasive biomarkers to characterize and monitor immune status in NSCLC patients treated with immunotherapy or chemoimmunotherapy.
Study supported by AECC Valencia Scientific Foundation, CB16/12/00350 and PI18/00266 (ISCIII) and AICO/2021/333 (GVA).

https://doi.org/10.1016/j.jlb.2024.100242

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PP01.63
The Journal of Liquid Biopsy 5 (2024) e1–e32 100241
Algorithmic genomic alteration filtering and Circulating Tumor DNA (CTDNA) quantification in serial Liquid Biopsy (LBX) from patients with prostateccancer

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Introduction: Several approaches exist to quantify ctDNA in LBx, with many using genomic alteration (GA) variant allele frequencies (VAFs) as a proxy for ctDNA tumor fraction (TF). GAs from non-tumor sources such as clonal hematopoiesis (CH) and germline can confound estimates. We evaluate the contribution of algorithmic filtering of non-tumor GAs to ctDNA quantification over time and the impact of CH on detection of targetable alterations.
Methods: Serial real-world LBx specimens from 337 patients with prostate cancer were analyzed. ctDNA TF on FoundationOne®Liquid CDx was quantified by aneuploidy and VAF of GAs, excluding CH using fragmentomics, as previously described (PMID: 38526394). A deep learning prediction model incorporating sequencing features was used for variant origin prediction. Maximum VAF (maxVAF) was iteratively calculated for each specimen, algorithmically excluding germline and CH variants. Fold change in maxVAF and ctDNA TF were compared with Spearman's coefficients.
Results: Median time between LBxs was 42 weeks (IQR: 19 – 72). The median patient age at first LBx was 72. For pathogenic GAs detected at baseline, see Table for the frequency of somatic, CH, and germline predictions. Correlation between change in maxVAF and ctDNA TF improved with each additional variant filter (All variants: 0.307; Excluding germline: 0.729; Excluding germline and CH: 0.904). All GAs classified as germline (n = 3,240) in the first specimen were detected in the second specimen. For GAs detected in both specimens, PPA was 99.6% and 93.4% (751/804) respectively for germline and CH classifications.
Conclusions: We demonstrate filtering of germline and CH is critical to quantify ctDNA change over time and that an algorithmic approach in the absence of paired PBMC sequencing is feasible. Additionally, algorithmic filtering identifies high rates of CH in a subset of actionable GAs in prostate cancer, highlighting the need to identify CH to avoid incorrect therapy selection.

Gene	Predicated Somatic	Predicted CH	Predicted Germline
All	311 (36%)	504 (59%)	40 (5%)
AR	49 (100%)	0 (0%)	0 (0%)
ASXL1	8 (15%)	46 (84%)	1 (2%)
ATM	1 (5%)	17 (85%)	2 (10%)
BRCA1	14 (82%)	1 (6%)	2 (12%)
BRCA2	6 (75%)	0 (0%)	2 (25%)
CHEK2	0 (0%)	15 (83%)	3 (17%)
DNMT3A	0 (0%)	198 (99.5%)	1 (0.5%)
PIK3CA	7 (100%)	0 (0%)	0 (0%)
PTEN	20 (100%)	0 (0%)	0 (0%)
SPOP	14 (0%)	0 (0%)	0 (0%)
TP53	73 (51%)	71 (49%)	0 (0%)

https://doi.org/10.1016/j.jlb.2024.100241

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PP01.64
The Journal of Liquid Biopsy 5 (2024) e1–e32 100242
Computational Variant Origin Prediction (VOP) to distinguish germline, tumor somatic, and Clonal Hematopoiesis (CH) variants in Liquid Biopsy (LBX)

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Introduction: CH is a premalignant acquisition of mutations in the bone marrow compartment that can be detected by LBx. These mutations can thus be mistaken for tumor-derived mutations, and can confound treatment decisions and clinical trial enrollment, especially when detected in therapeutic targets (ATM and CHEK2 for PARP inhibitors, TP53 for novel agents under investigation).
Methods: Paired LBx and buffy coat from 1977 pan-cancer patients were sequenced using the FoundationOne®Liquid CDx platform. We trained a deep learning prediction model that incorporates fragmentomics and other sequencing features to assign three probabilities of variant origin and established a categorization threshold maximizing Youden's index. We assessed performance of VOP by crossfold validation in 359 patients with 3 samples available: LBx, buffy coat, and tissue biopsies sequenced with FoundationOne®CDx.
Results: 559 pathogenic CH variants were detected in 248/359 patients (69%).