Immune infiltrate co-occurrence and neoantigen similarity are prognostic factors in early-stage NSCLC

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BACKGROUND

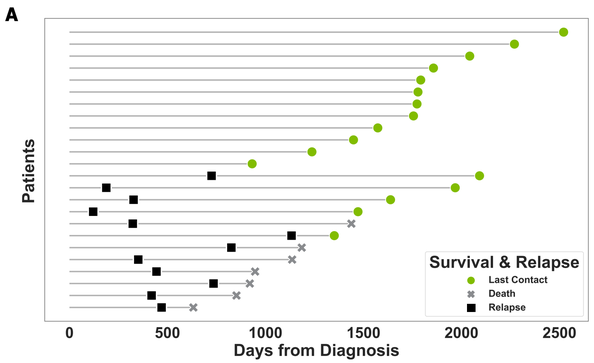
The prevalence of early-stage non–small cell lung cancer (NSCLC) with curative treatment options is expected to increase with the recent implementation of annual screening programs. Predictors and molecular drivers of disease relapse, especially the role of intra-tumoral immune dysfunction, remains unclear but critical for the refinement of therapeutic decisions. By leveraging a comprehensive individual portrait of each patient's immune system, potential novel mechanisms associated with tumor relapse in early-stage NSCLC may be identified.

METHODS

Patients and platform

The ImmunoID NeXT platform® was used to profile 20 (**A**) stage IA-IIIB lung adenocarcinoma patients who underwent curative treatment. The group consisted of 10 non-relapsed (at least 2 year follow-up) patients and 10 covariate-matched ci (gender, age, stage) relapsed patients. ImmunoID NeXT (**B**) outputs leveraged in our analyses include variant and CNA calling, gene expression quantification, HLA profiling (typing, mutation, and loss of heterozygosity), T-cell receptor and tumor microenvironment (TME) profiling, and neoantigen prediction. ImmunoID NeXT applies SHERPA® (Systematic HLA Epitope Ranking Pan Algorithm), a pan-allelic MHC-peptide algorithm to predict MHC-peptide binding and presentation[1]. Neoantigen peptide sequences were subjected to further filtering and clustering based on between-patient similarity scores, with the goal of identifying shared clusters of relapse-associated neoantigens in each possible patient pair. Differential network analyses were applied to the TME composition estimates to investigate relapse-associated patterns of cellular co-occurrence and interaction.

A



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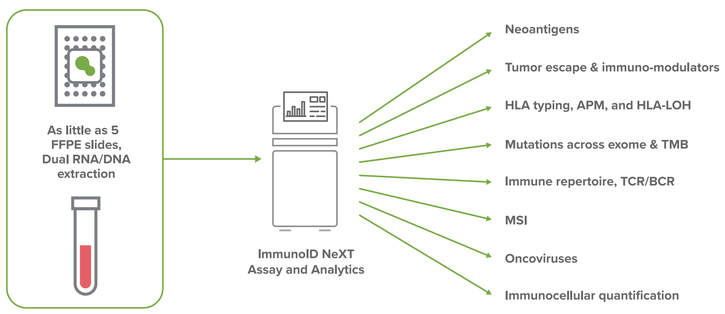
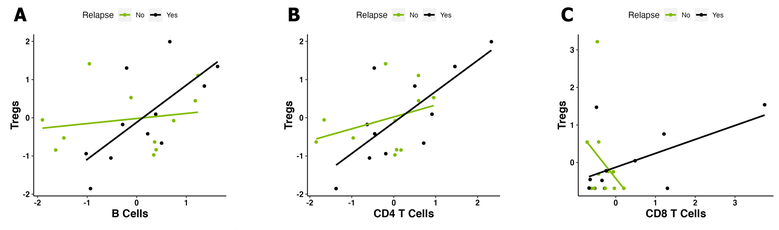


RESULTS

Suppressive immunity in the tumor microenvironment

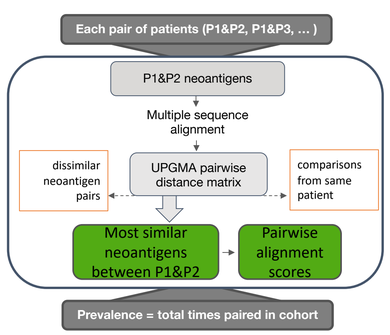
In the TME, we observed similar immune cell compositions between relapsed and non-relapsed patients. However, we found a positive correlation between Tregs and both B (**A**) and CD4 T cells (**B**) in relapsed patients, indicating suppressive anti-tumor immunity (Pearson's R=0.7 and 0.74, both P<0.02 vs. R=0.18 and 0.35, both P>0.2 in non-relapsed patients). Of note, CD8 T cells and Tregs (**C**) tended to co-exist in relapsed patients, while a mutually exclusive/negative correlation was observed in non-relapsed patients ($P_{interaction} < 0.05$).



Neoantigen prevalence and binding rank

For each pair of neoantigens within each pair of patients, a BLOSUM-matrix-based multiple sequence alignment was used to generate a UPGMA distance matrix between amino-acid sequences. We then discarded pairs that were above a distance threshold to derive a subset of the most similar neoantigens between each pair of patients. An alignment score was then generated for each pair of neoantigens passing the threshold. For each neoantigen, we established a prevalence metric based on the number of times the neoantigen was matched with any other neoantigen in the cohort (**A**). When considering neoantigens selected on the basis of alignment score, we found that those belonging to non-relapsed patients had significantly lower HLA binding ranks compared to those of relapsed patients. SHERPA binding rank comparison of all neoantigens for reference (**B**). The 3 highest scoring (**C**) or single-highest scoring (**D**) neoantigens per patient among those found in expressed genes. The mean non-relapse minus relapse binding ranks are shown below each plot. Mixed-linear model p values shown. This result implies that filtering neoantigens based on high interpatient similarity may define a representative set with biological importance.

A



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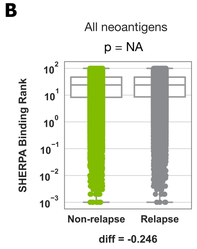

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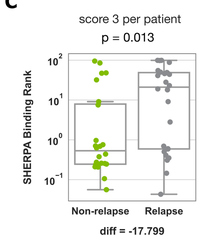

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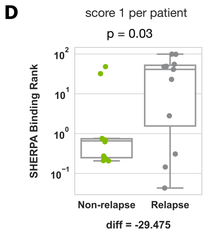
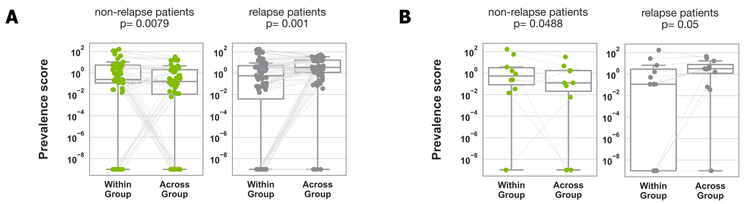


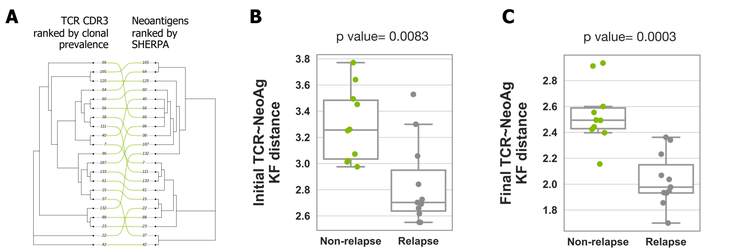
Neoantigens among non-relapsed patients are more similar to one another than those of relapsed patients

To assess whether neoantigen similarity correlates with relapse, we selected either the 3 highest scoring (**A**) or single highest scoring (**B**) neoantigens per patient. A prevalence score was calculated based on the number of pairings within or across relapse groups, normalized to the possible number of pairings. Among non-relapse patients, neoantigens tended to be paired with other non-relapse patients more than with relapse patients (Wilcoxon p values shown).



Optimized distance between TCR CDR3 and neoantigen clusters correlates with relapse

We next assessed the relative similarity between neoantigen and TCR diversity. For each patient, the 45 neoantigens with the highest SHERPA ranks, and the TCR CDR3-beta sequences of the most prevalent 45 TCR clones, were each clustered using maximum parsimony (MP). An initial Kuhner-Felsenstein (KF) distance[2] was calculated between each neoantigen and TCR tree (**A**), and then rank orders of TCR were optimized using a random swap for 10,000 iterations. Both initial (**B**) and optimized (**C**) KF distances were significantly lower for relapsed patients, implying a more tightly matched clustering structure between the most prevalent TCR and those neoantigens most likely to be presented (Mann-Whitney p-values shown).



CONCLUSION

In this pilot cohort, we used an integrated platform to broadly characterize both the tumor and immune system, enabling identification of relapse-associated neoantigens that may share universal features. Relapse in early-stage LUAD patients was associated with an immunosuppressive TME and weak binding among similar neoantigens, but a well-matched diversity of T cell receptors. Combined, these results suggest that the relevant T cells are present in the tumors of relapsing patients, but less effective. These findings demonstrate that deep profiling of shared neoantigen features has the potential to become an early biomarker of relapse, informing patient therapy selection and surveillance.

Reference:

1. Pyke RM, Mellacheruvu D, Dea S, Abbott CW, Zhang S v., Phillips NA, et al. Precision neoantigen discovery using large-scale immunopeptidomes and composite modeling of MHC peptide presentation. Molecular and Cellular Proteomics. American Society for Biochemistry and Molecular Biology Inc.; 2021;20.
2. Kuhner, M. K. and Felsenstein, J. (1994) Simulation comparison of phylogeny algorithms under equal and unequal evolutionary rates. Molecular Biology and Evolution, 11, 459–468.