Single-cell analysis of peripheral blood mononuclear cells reveals therapy outcomes are associated with pre-existing immunity in patients treated with oncolytic adenovirus armed with TNFα and IL2

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Background:
Engineered oncolytic adenovirus Ad5/3-E2F-d24-hTNFa-IRES-hIL2 TILT-123 has emerged as a promising candidate for clinical translation due to its ability to induce tumor regression and activate host immunity. As a result, the phase 1 clinical trial TUNIMO (NCT04695327) assessed the safety of TILT-123 in patients with advanced solid tumors and provided important insights into its therapeutic efficacy. This study aimed to identify immunological markers in peripheral blood capable of predicting treatment outcomes and to elucidate the mechanisms through which TILT-123 modulates immune responses in these patients.

Methods:
Single-cell RNA sequencing was performed on peripheral blood mononuclear cells (PBMCs) collected from patients with various solid tumors at baseline and after TILT-123 administration on day 64. The BD Rhapsody platform was used for cell capture and library preparation. Data processing, including alignment, quantification, and quality control, was carried out using R. Differential gene expression analysis and immune cell population profiling were performed to identify changes in immune cell composition and activation states. These findings were then correlated with available clinical data.

Results:
Patients with better outcomes exhibited higher baseline levels of cytotoxic effector cells, such as CD8+ T cells, alongside an increased presence of CD16+ monocytes and a reduced proportion of regulatory T cells, compared to those with poorer outcomes. TCR repertoire analysis revealed greater diversity and clonal persistence in patients with better outcomes, with significant correlations observed between specific TCR profiles at baseline and overall survival. In contrast, patients with poorer outcomes demonstrated impaired NK cell function and reduced inflammatory responses, contributing to a less favorable immune environment. Additionally, TILT-123 treatment enhanced B cell differentiation, promoted diversification of both B and T cell receptor repertoires, and supported the formation of immunological memory.

Conclusion:
Our findings suggest that pre-existing immunity and favorable immune profiles at baseline are associated with better outcomes in response to TILT-123. These results

support the potential of personalized treatment strategies to enhance therapeutic efficacy.