184 DISCOVERY, CLONING AND FUNCTIONAL VALIDATION OF A NEOANTIGEN SPECIFIC PATIENT DERIVED TCR ON THE BERKELEY LIGHTS PLATFORM, WITH IMPLICATIONS IN PERSONALIZED CANCER IMMUNOTHERAPY

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Background Pancreatic ductal adenocarcinoma (PDAC) has the poorest prognosis of all human cancers and requires new therapeutic options. Several immune-based treatments including personalized neoantigen vaccines and adoptive T cell transfer are currently being investigated. Recently, complete clinical response was reported in a patient with HER2+ metastatic PDAC treated with a neoantigen vaccine in combination with radiation and dual checkpoint (https://doi.org/10.1101/2021.12.16.21267326). Here we describe a sensitive method for post-vaccination functional characterization of neoantigen-specific T cells from minimal quantity of patient peripheral blood at the single cell level using a microfluidic device on the Beacon® optofluidic system from Berkeley Lights.

Methods Peripheral blood was obtained from a patient with metastatic PDAC in complete response following neoantigen DNA vaccine therapy. T cells were pre-enriched by stimulating with a cocktail of peptides corresponding to 7 neoantigens. Subsequently, single T cells were investigated over 2 days using a microfluidic device featuring nanoliter-scale NanoPen® chambers in co-culture with autologous dendritic cells or partially HLA-matched allogeneic antigen presenting cells (APCs) pulsed with the neoantigen peptides. Antigen-responsive T cells were isolated based on expression of CD137, a known T cell activation-associated marker, cytokine secretion and cytotoxicity profile. TCRs of selected T cells were recovered, synthesized, cloned into a lentiviral expression vector, expressed in primary T cells from an unrelated donor, and validated using peptide pulsed APCs.

Results A quantitative dataset including CD137 expression, IFN-y secretion and real time cytotoxicity was generated for single T cells from the peripheral blood of a cancer patient in co-culture with single APCs. Candidates were ranked based on strength of phenotype, isolated and TCR sequences were generated. The top TCR candidate, restricted to HLA-A*02:01, post-engineering was effective in recapitulating the same potency criteria (99% cytotoxicity, 74% CD137+ as compared to <1% for controls and >15,000 fold enrichment of IFNy/TNFa/IL-2 as compared to controls) against a lymphoma derived cell line presenting a single peptide from the cancer vaccine. Importantly, this TCR was not active against the other neoantigen peptides or a well characterized oncogenic peptide MART-1 demonstrating specificity.

Conclusions We demonstrate the ability to characterize T cells in co-culture with APCs at single cell resolution. A TCR which exhibited strong functional behavior during screening was also effective and specific to a single neoantigen peptide post engineering. This workflow may enable personalized TCR to be realized by reducing the timeframe and finding better candidates to be used independently or in combination with cancer vaccines and other interventions.

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