A High-throughput Platform To Produce NeoE-HLA Libraries For Capturing NeoE-specific T Cells From Peripheral Blood Of Patients With Solid Tumors

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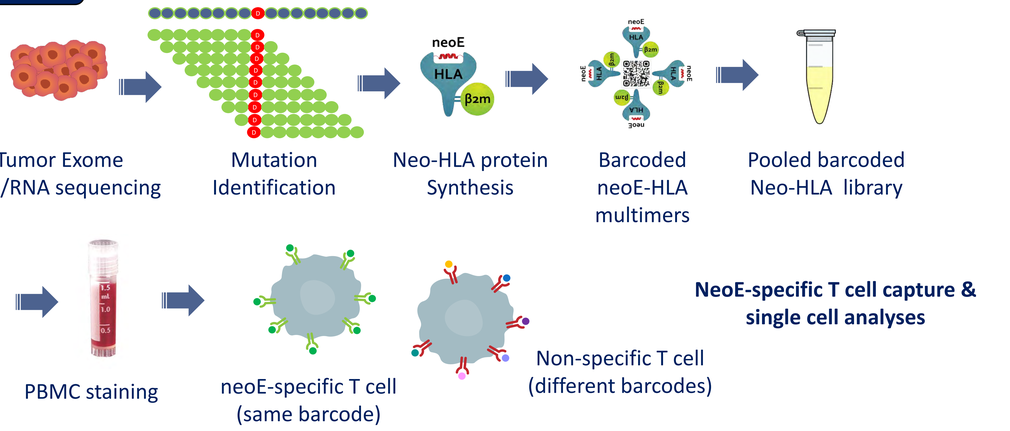
Abstract

We have developed a high-throughput, automated process for the cloning, expression and purification of soluble proteins comprising neoE peptide with beta2m and the HLA heavy chain fused into a single polypeptide (neoE-HLA proteins). Of the 13 thousand HLA alleles in the human population, with 6 HLA alleles expressed in each person, our HLA catalog enables rapid production of neoE-HLA protein candidate libraries, representing >4 of 6 HLAs coverage for ≥95% of all patients, regardless of ethnicity. We can generate neoE-HLA libraries in single production runs for up to several hundred neoE-HLA candidates per patient. These soluble neoE-HLA proteins are then assembled into barcoded "snare libraries" for the interrogation of matched PBMCs from that patient for CD8 T cells that specifically bind the cognate neoE-HLA tumor targets. The imPACT Isolation Technology® is capable of capturing CD8 T cells of a frequency as low as 1 per 10^6.

This platform is capable of producing snares predicted to represent high affinity (≤500 nM) and low affinity (>500nM) neoE binding to their corresponding HLAs. These capabilities permit deep and broad interrogation of the tumor-targeted neoE-specific T cell repertoire in the blood of patients with solid cancers as exemplified here. In summary, our proprietary neoE-HLA prediction pipeline and the proprietary high-throughput neoE-HLA protein production platform enables the discovery of tumor-targeted, neoE-specific T cells from patients. These breakthrough technologies support the on-going Phase 1 clinical trial of personalized engineered autologous NeoTCR-P1 T cell therapies for patients with eight different solid tumor types (NCT03970382).

Methods



Conclusions

- Modular NeoE-HLA libraries across a broad spectrum of HLAs holds potential for NeoE-specific T cell capture from >99% of all individuals with cancer, regardless of HLA-type/ethnicity or tumor type.
- Bioinformatic analysis and prediction is used to generate dozens-to-several hundred unique NeoE-HLA protein "snares" targeting each patients private tumor mutations. Machine learning from results obtained has potential to improve neoepitope predictions across the HLA spectrum.
- Capturing rare pre-existing NeoE-specific T cells authenticates tumor-exclusive neoantigen mutation targets for developing personalized therapies and for immune monitoring of patients with cancer.
- Phase 1 clinical trial of personalized engineered autologous NeoTCR-P1 T cell therapies is on-going for patients with 8 solid cancer types.

Results

NeoE-HLA protein engineering supports high throughput production of patient-specific barcoded "snare libraries"

1) Modular: NeoE, HLA, and β2M are covalently linked into a single polypeptide.
2) This approach is applicable to the spectrum of HLA alleles.
3) Our current catalog of HLA alleles covers >95% of the population with 4 of 6 alleles per person
4) Automation supports production of hundreds of candidate patient-specific NeoE-HLA proteins in several days.

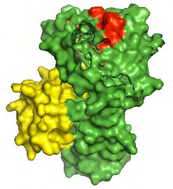
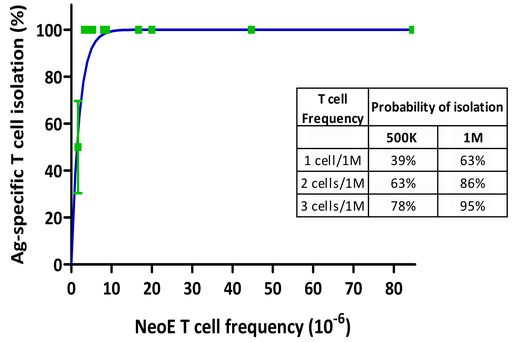


Figure 1. Design of neoE-HLA soluble proteins. NeoE (red), β2m (yellow) and HLA heavy chain (green).

Capture of rare CD8 T cells is highly sensitive & reproducible



T cell Frequency	Probability of isolation	
	500K	1M
1 cell/1M	39%	63%
2 cells/1M	63%	86%
3 cells/1M	78%	95%

Figure 2. Sensitivity & reproducibility of antigen-specific T cell capture. Three independent experiments are shown using blood from same donor to capture CMV-specific T cells. Blue curve: theoretical probability of detecting at least 1 target by sampling 500K events, as a function of target frequency. Green dots: experimental data from process reproducibility tests.

NeoE-specific T cells are captured across a wide spectrum of HLAs and predicted binding affinities

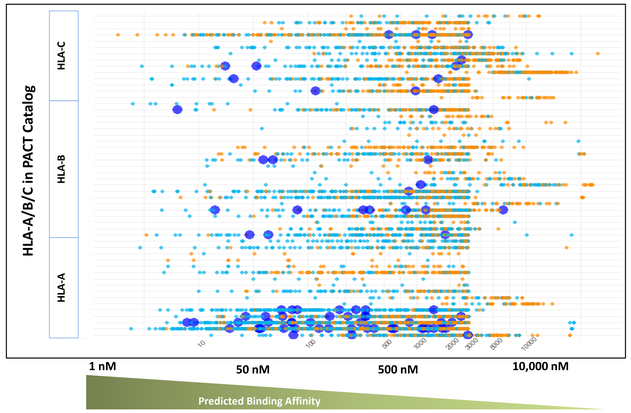


Figure 3. NeoE-HLA protein candidates translate into neo-T cell capture from the blood of patients with cancer.
From 68 patients with cancer, ~14K neoE-HLA candidates (small cyan and orange dots) were predicted across HLA types. Predicted NeoE-HLA binding affinities are shown on the x axis. NeoE-HLA candidate proteins that could be produced are shown in cyan. Predicted neo-HLA protein candidates that did not fold correctly are shown in orange. Big dark blue dots represent neoE-HLA proteins that led to the capture of rare neoE-specific T cells from patient peripheral blood.

Isolation of rare T cells with known neoantigen specificity from blood

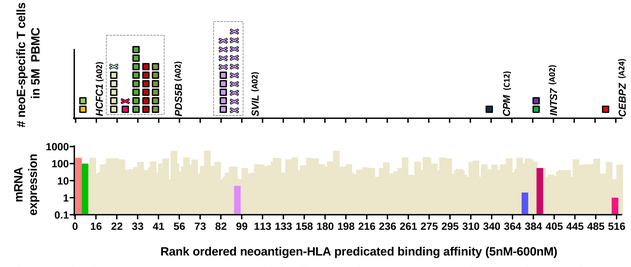


Figure 4. Capture of neoE-specific T cells from blood of a patient with breast cancer. (Bottom) mRNA expression and predicted neoE-HLA binding affinity. In total, 262 neo-HLA protein molecules covering 107 mutations were synthesized. Colored bars indicate neoE-HLAs for which cognate T cells were captured. (Top) neoE-specific T cells captured in ~5mL blood. Each box depicts 1 T cell, while **X** indicates 10 T cells. Each color represents a unique T cell receptor sequence/clonotype.