Artificial Immune Modulation (AIM) nanoparticles expand antigen specific CD8 T cells from both naïve and memory T cell populations

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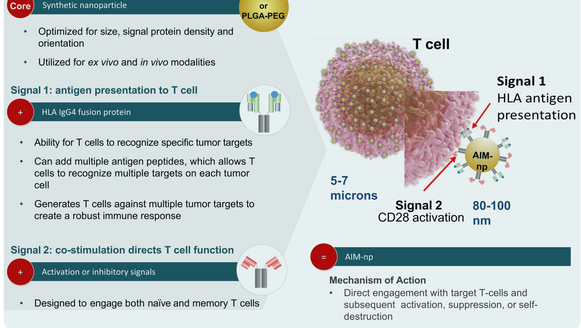
Introduction

NexImmune is developing a novel approach to immunotherapy designed to employ the body's own T cells to generate a specific, potent, and durable immune response by utilizing a proprietary Artificial Immune Modulation (AIM™) nanoparticle technology platform. AIM constructed nanoparticles (AIM-np) function as synthetic dendritic cells capable of directing the immune function of antigen-specific T cells. AIM-np employ natural biology to engage, activate, and expand endogenous T cells in ways that combine the key anti-tumor attributes of antigen-specific precision, potency, and long-term persistence with reduced potential for off-target toxicities. In this study, we used single cell sequencing based on the 10X Genomics platform to explore the clonotypes of AIM ACT cell products expanded from healthy donor T cells using either MART1-peptide loaded AIM nanoparticles (np's) or a cocktail targeting 6 EBV peptides simultaneously.

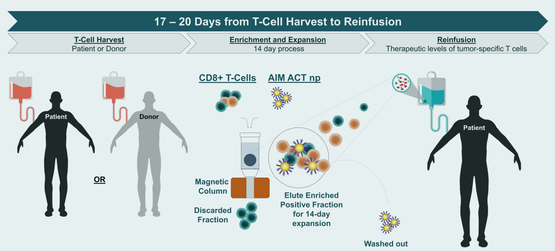
AIM Platform

Artificial Immune Modulation (AIM™) nanotechnology platform



AIM ACT (Adoptive Cell Therapy)

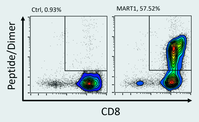
Autologous or allogenic ACT for targeted tumor treatment



Fully-closed and scalable manufacturing process using AIM-np to enrich and expand (E+E) CD8⁺ T cells for adoptive cell therapies that have demonstrated pharmaceutical precision.

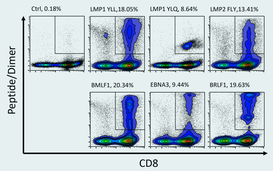
T Cell Specificity

E+E process generates highly antigen-specific T cells



Antigen	Specificity (%)
MART1	57.52
Ctrl	0.93
Total Specificity	56.59

The final MART1 T cell products include about 56.6% MART1-specific CD8+ T cells as determined by MART1 peptide (ELAGIGILTV)-loaded multimer staining.

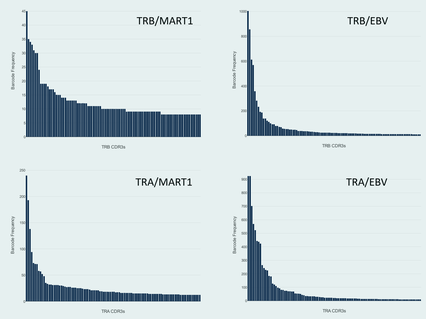


Antigen	Specificity (%)
BMLF1	20.34
BRLF1	19.63
EBNA3	9.44
LMP1 YLL	18.05
LMP1 YLQ	8.64
LMP2 FLY	13.41
Ctrl	0.18
Total Specificity	88.43

The final EBV T cell products include about 88.4% EBV antigen-specific CD8+ T cells as determined by peptide-loaded multimer staining. BMLF1, GLCTLVAML. BRLF1, YVLDHLIVV. EBNA3, LLDFVRFMGV. LMP1 YLL, YLLEMLWRL. LMP1 YLQ, YLQQNWWTL. LMP2 FLY, FLYALALLL.

Clonotype Distribution

Distinct CDR3 abundance and clonotype distribution between MART1 and EBV AIM ACT T cells



Clonotypes	% Top 10 in total	% Top 100 in total	Total sequenced
MART1 AIM ACT	1.82	7.92	11,936
EBV AIM ACT	43.87	73.12	9,236

AIM ACT cells were sequenced with Chromium Single Cell Immune Profiling (10X Genomics) to analyze full-length V(D)J sequences for paired T-cell receptors. Left, CDR3 abundance for MART1 AIM ACT. Right, CDR3 abundance for EBV AIM ACT.

V-J Gene Heatmap

AIM ACT MART1 TCR heavily use TRAV12-2

AIM ACT MART1 T cells heavily use TRAV12-2 in their TRA chains, which is consistent with the finding from many other publications. TRA chains containing TRAV12-2 account for 67.37% of total sequenced cells (8,041 out of 11,936). There is no identifiable pattern for AIM ACT EBV T cells in VDJ usage.

Mapping to Known TCRs

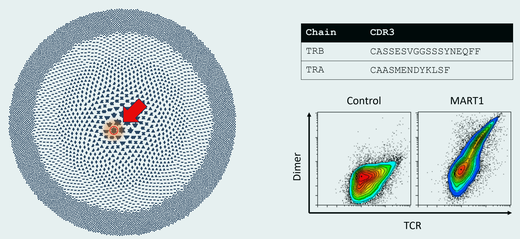
CDR3 mapping to known TCR database

CDR3 matching	Antigen protein	Cell number	% of total sequenced
MART1 AIM ACT	MART1	167 / 11,936	1.40
EBV AIM ACT	LMP2, BMLF1, BRLF1	758 / 9,236	8.21

CDR3 sequences from TRB were used to query Pathology-associated TCR database (http://friedmanlab.weizmann.ac.il/McPAS-TCR/, Weizmann Institute of Science), the cell number and % of total sequenced cells with matching TRB CDR3 were listed.

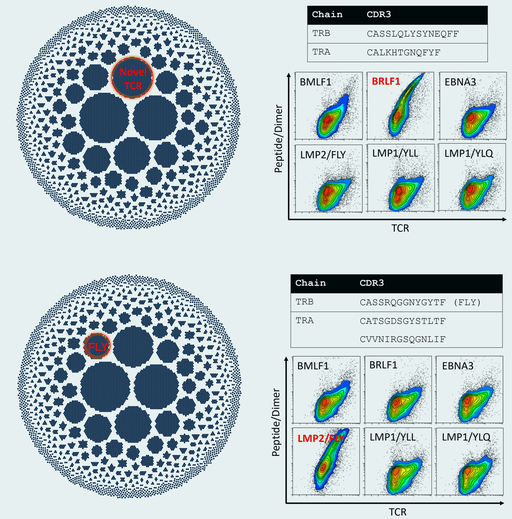
Novel TCR Screening

Verification of newly identified TCRs by multimer staining



3 representative previously unknown full TCR sequences from Single Cell Immune Profiling containing paired TRA and TRB chains were cloned into mammalian cell expressing plasmids and transient transfected 293F/CD3 cells. Transient expressed TCRs were then stained with MART1 peptide (ELAGIGILTV)-loaded multimer staining and confirmed to be MART1 specific. One representative staining shown.

EBV TCR verification by multimer staining



Previously described and newly identified full TCR sequences from Single Cell Immune Profiling containing paired TRA and TRB chains were cloned into mammalian cell expressing plasmids and transient transfected 293F/CD3 cells. Transient expressed TCRs were stained with EBV peptide antigen-loaded multimers to confirm their specificity. In total 10 pairs of representative TCRs from 6 clonotypes were tested and confirmed each clonotype to be specific to one of EBV specific peptide antigens.

Conclusion

The clonotype distribution was significantly different between MART1 E+E cells and EBV E+E cells. The top 10 clonotypes from MART1 E+E cells accounted for less than 2% of total TCRs sequenced, whereas the top 100 clonotypes from MART1 E+E cells accounted for 8% of total TCRs sequenced. On the other hand, the top 10 clonotypes from EBV E+E cells accounted for about 44% of total TCRs sequenced, whereas the top 100 clonotypes from EBV E+E cells accounted for 73% of total TCRs sequenced. About 95% of the human population were EBV positive thus most healthy donors already have an EBV-specific memory T cell population while in contrast, healthy donors have about 1000-fold lower T cell precursor frequencies for MART1, and these were typically of the naïve phenotype. Therefore, the clonotype difference between MART1 and EBV T cell expansion using NexImmune's AIM ACT aAPC may be related to progenitor populations in respective donors, suggesting that AIM ACT nanoparticles expand antigen-specific CD8 T cells from both naïve and memory T cell populations. Overall, the single cell sequencing results demonstrated that NexImmune's AIM platform expands broad spectrum of multiclonal, highly antigen-specific CD8 T cells for adoptive cell therapy. The AIM ACT platform also opens opportunities for antigen-specific TCR screening of interest.

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