Conversion of peripheral blood mononuclear cells into tumor-specific cytolytic cell populations using tumor cells engineered with multiple immunomodulatory factors

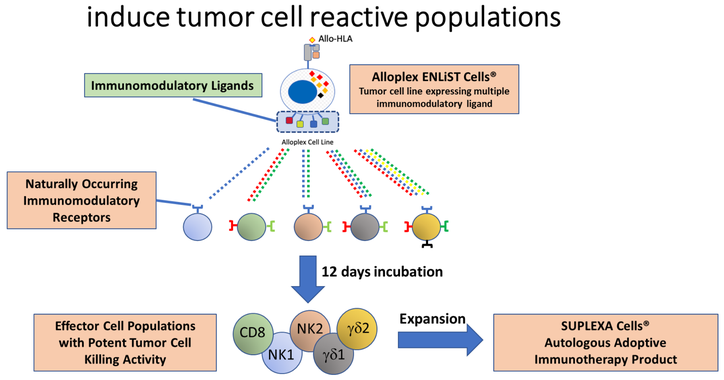
James Lederer, PhD[1,2] , Joshua Keegan, BS[1,2], Nathan Schomer, BS[1], Frank Borriello MD, PhD[1]
1, Alloplex Biotherapeutics, Inc; 2, Brigham and Women's Hospital and Harvard Medical School

Background

Numerous studies and two recent clinical approvals have demonstrated the efficacy and safety of cellular immunotherapies to treat diverse cancer subtypes. To date, the only approved cytotoxic cellular therapies in the U.S. are T-cell based. However, ongoing clinical trials using γδ T cells and NK cells have suggested not only clinical efficacy, but also synergistic beneficial effects when combined with checkpoint inhibitor antibody therapies. We have developed novel immune stimulatory allogeneic tumor cells engineered with multiple immunomodulatory factors as an approach to generate heterologous tumor cell lytic populations for human peripheral blood mononuclear cells (PBMCs) for cellular immunotherapy. Here, we present our findings on converting normal PBMCs into potent tumor cell lytic population and identify their single-cell phenotypes using CyTOF mass cytometry technology

Graphical Hypothesis

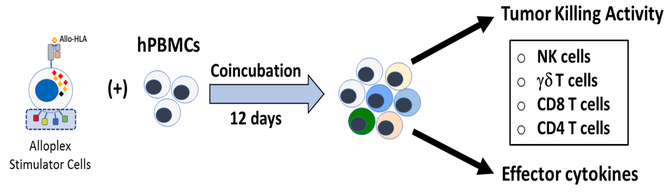
Engineered Leukocyte Stimulator (ENLiST®) cells activate multiple natural immune recognition receptors on human PBMCs to induce tumor cell reactive populations



Methods

SK-MEL-2 cells (NIH) were engineered using lentivirus to express of immunomodulatory proteins (>15) with the to expand tumor cell reactive immune cell populations. Engineered tumor cells were tmixed with freshly thawed human PBMCs from healthy volunteers and co-incubated for up to 12 days. Cells were collected at time points during the culture period for phenotypic analysis mass cytometry (CyTOF). Functional characterization of the converted cells was performed by cytotoxicity assay against targets from several cancer subtypes

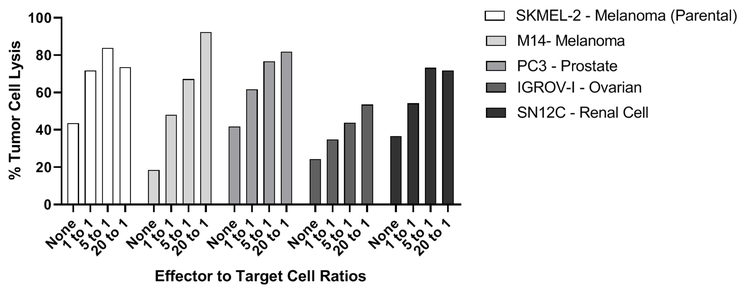
Mixed Lymphocyte-Tumor Cell Reaction (MLTR) to generate tumor killing immune cell subsets from normal human PBMCs



- ✓ *induces hPBMC activation and proliferation*
- ✓ *induces hPBMC differentiation*
- ✓ *induces hPBMC cytokine production*
- ✓ *induces mixtures of tumor killing immune cell subsets*

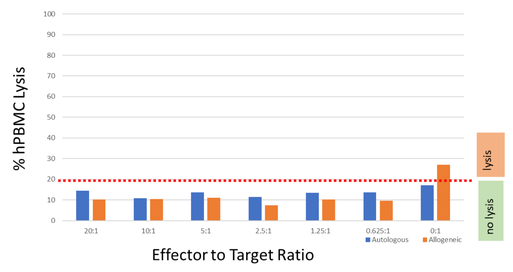
Results

Figure 1: Cytotoxicity assays measuring tumor killing activity of ENLiST cell® 12 day differentiated hPBMCs



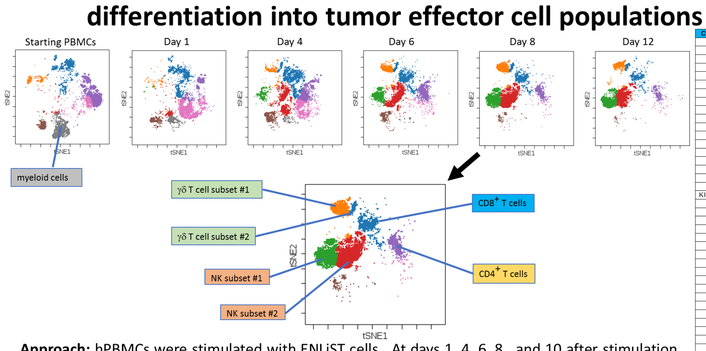
Approach: Tumor killing assays were done at 1 day after mixing differentiated PBMC populations with Cell Trace Violet labeled target cells at the indicated effector to target cell ratios. Killing was measured by flow cytometry.

Figure 2: Differentiated hPBMC effector cells do not kill autologous or allogeneic human PBMCs



Approach: Human PBMCs were labeled with Cell Trace Violet and used a autologous or allogeneic targets in killing assays using 12 day EMLiST cell stimulated hPBMC as effector cells.

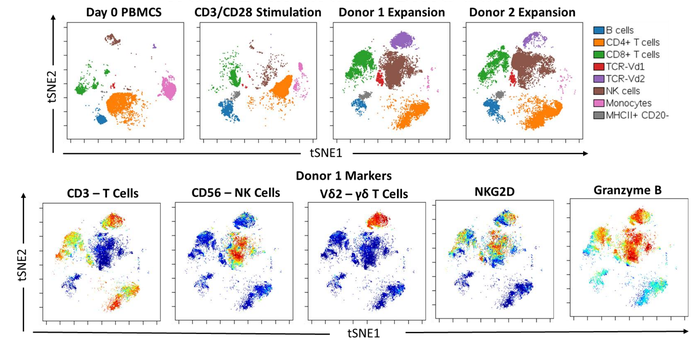
Figure 3: CyTOF analysis of ENLiST cell driven hPBMC differentiation into tumor effector cell populations



CyTOF Antibody Panel	
Marker	Metal
CD45	89Y
CD14	111Cd
CD20	113In
CD3	115In
Granulysin	141Pr
Ki67	142Nd
PD-1	143Nd
CD39	144Nd
NKp44	145Nd
TCRγδ	146Nd
CD45RO	147Sm
Granzyme A	148Nd
Granzyme K	149Sm
TIM-3	150Nd
TCR Vδ1	151Eu
CTLA-4	152Sm
CD69	153Eu
KIR2DL4/CD158d	154Sm
CD4	155Gd
CD8b	156Gd
CD16	157Gd
CD8a	158Gd
CD57	159Tb
NKp30	160Gd
PSGL1	161Dy
CD56	162Dy
CD1c	163Dy
CXCR6	164Dy
GRAIL	165Ho
CD107a	166Er
Grz B	167Er
NKp46	168Er
CD3z	169Tm
CD127	170Er
TCR Vδ2	171Yb
NKG2D	172Yb
CD25	173Yb
CD158b2	174Yb
Tbet	175Lu
Perforin	176Yb
HLA-DR	209Bi

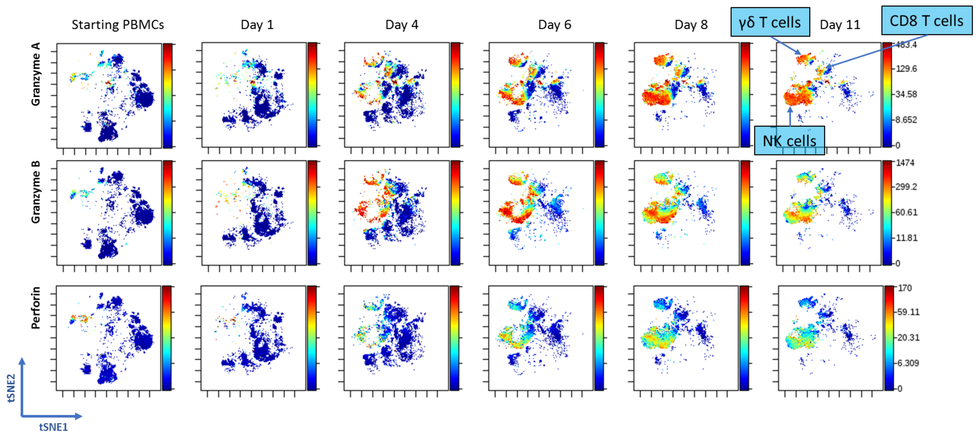
Approach: hPBMCs were stimulated with ENLiST cells. At days 1, 4, 6, 8 , and 10 after stimulation, cells were harvested and stained with a 39-marker CyTOF panels to detect multiple immune cells subsets. Data was analyzed by viSNE with cell identifying marker overlays presented here.

Figure 4: CyTOF analysis of ENLiST cell® stimulated hPBMCs from 2 different PBMC donors



Approach: Differentiated cells from 2 donors were analyzed by CyTOF to identify immune cell subsets and functional markers. ENLiST activation and differentiation was compared to anti-CD3/CD28 stimulated hPBMCs.

Figure 5: ENLiST cell stimulation induces functional tumor cell killing molecules in NK cells, γδ T cells, and CD8 T cells



Results and Conclusions

These findings indicate that human tumor cell lines that express multiple immunostimulatory can induce potent tumor killing immune cell populations with broad tumor cell reactivity but no detectable killing of allogeneic PBMCs. This strategy is being developed for clinical use as an autologous adoptive immunotherapy for cancers. We report that:

1. ENLiST cell stimulated and differentiated hPBMCs develop into tumor killing populations with potent activity against multiple types of tumor cells
2. ENLiST differentiated hPBMCs do not kill autologous or allogeneic PBMC, which suggests that they will not kill normal/non-tumor cells
3. CyTOF analysis provides a clear picture of the immune effector cell types that arise in these ENLiST cell differentiation cultures. These cells also acquire increased expression of functional effector factors such as Granzymes and Perforin