A Differentiated and Broadly Applicable Autologous Whole Blood Derived Adoptive Cellular Therapy For Treating Cancer

Frank Borriello, MD, PhD*[1], Joshua Keegan, BS[1], and James Lederer, PhD[1,2]

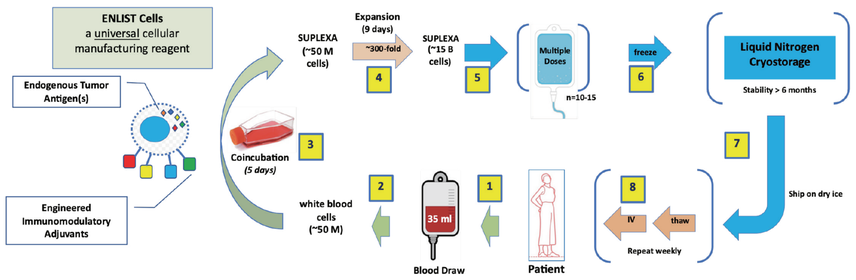
[1]Alloplex Biotherapeutics, Inc., Woburn, MA, [2]Department of Surgery, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

*Presenter and Corresponding Author, fborriello@alloplexbio.com

Background

Alloplex Biotherapeutics has developed a novel cellular therapeutic that uses ENgineered Leukocyte ImmunoSTimulatory cell lines called **ENLIST** cells to activate and expand a heterogeneous population of tumor killing effector cells from human peripheral blood mononuclear cells (PBMCs). This process results in a 300-fold cellular expansion containing NK cells, CD8+ T cells, γδ T cells, TCR variant NKT-like cells and CD4+ T cells, collectively called **SUPLEXA** therapeutic cells.

The SUPLEXA cells manufacturing process uses peripheral blood mononuclear cells (PBMCs) from cancer patients. PBMCs are then stimulated with ENLIST cells for a 5-day induction period, which is then followed by a 9-day cytokine-induced expansion period. SUPLEXA cells are then cryopreserved for later use as an autologous adoptive immunotherapy. A first-in-human clinical trial for this novel adoptive cellular therapeutic for cancer is projected to begin later this year.



Methods

ENLIST cells: engineered SK-MEL2 melanoma cell line that expresses curated sets of > 20 different immunomodulatory proteins that are engineered for membrane expression.

SUPLEXA and cytokine-induced killer (CIK) cell manufacture: PBMCs from normal healthy volunteers (NHVs) were co-incubated with freeze/thaw dead ENLIST cells for 5 days followed by expansion in culture medium containing cytokine support. After 9 days, SUPLEXA cells were harvested and cryopreserved. Control cytokine induced killer (CIK) cells were prepared by culturing PBMCs for 9 days with media containing cytokine support only.

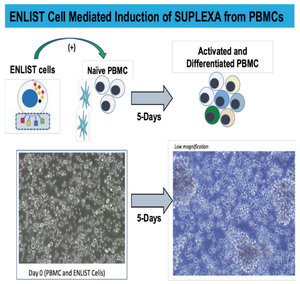
Mass Cytometry (CyTOF): Original PBMCs and matched SUPLEXA or CIK cells were comprehensively characterized by mass cytometry (CyTOF) using a 48-marker antibody panel. CyTOF data analysis was done using OMIQ for dimensional reduction by opt-SNE and cell subset phenotyping.

Tumor Cell Killing Assay: Tumor cytolytic activity was measured by flow cytometry using fluorescent tumor cell targets at 2:1, 1:1, and 1:2 effector:target cell ratios. M14 melanoma cells that express red fluorescent protein (RFP) were used as tumor cell targets for these studies.

Cytokines: A 33 cytokine Luminex panel was used to assess cytokine levels in cytolysis supernatants.

Results

Figure 1: SUPLEXA Cell Manufacturing. ENLIST immunomodulatory cells are mixed with PBMCs and cultured for 5 days to activate PBMCs. Activated cells are then expanded for 9 days in IL-7 and IL-15. Photomicrographs of 5-day activated PBMCs are shown along with a table listing broad tumor cell killing activity.



Qualitative Tumor Cell Killing Survey

Unmodified Cell Lines Used in Secondary Effector Assay	PBMC coincubated with *Unmodified SK-MEL-2* (% killing)	PBMC coincubated with *ENLIST Cells* * (% killing)
Melanoma Cell Lines		
• SK-MEL-2	0%	>95%
• M14	0%	>95%
• SK-MEL-28	0%	>95%
Non-Melanoma Cell Lines		
• COLO205 - colorectal	0%	>95%
• HOP62 - lung	0%	>95%
• IGR-OV1 - ovarian	0%	>95%
• SKOV - ovarian	0%	>95%
• PC3 - prostate	0%	>95%
• SN12C - renal	0%	>95%
• K562 - leukemia	0%	>95%
Autologous Leukemia Cells	0%	>95%

Quantitative Tumor Killing Assay

E:T ratio	melanoma M14	prostate PC-3	leukemia K652	colorectal COLO
>16:1				
16:1				
8:1				
4:1				
2:1				
1:1				
1:2				
1:4				

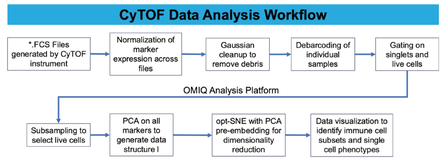
Interpretation:
- The induced cytolytic activity is broad and extends beyond melanoma, suggesting **HLA independence**
- **All tumor cell lines tested to date are susceptible**
- SUPLEXA cells do not kill each other or healthy PBMC

Figure 2: SUPLEXA and CIK Phenotyping By CyTOF – Antibody Panel and Data Analysis Workflow. SUPLEXA and cytokine-induced killer (CIK) cells were analyzed to compare single-cell phenotypes using a customized 48-marker CyTOF antibody panel to identify NK cell and T cell subsets as well as transcription factors, adhesion markers, and functional molecules. CyTOF antibody panels, CyTOF data analysis workflow, and cell subset overlay plots are shown here.

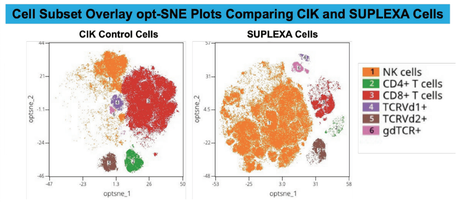
CyTOF Antibody Panel for SUPLEXA Characterization			
Marker	**Antibody Clone**	**Metal**	**Role**
CD45	HI30	89Y	Pan-leukocyte marker
CD172ab	SE5A5	111Cd	Myeloid cell marker
CD8a	RPA-T8	112Cd	CD8 T cell alpha-chain
CD20	2H7	113In	B cell marker
CD4	RPA-T4	114Cd	CD4 T cell marker
CD3	UCHT1	115In	All T cell marker
DNAM1	DX11	116Cd	Adhesion molecule for NK cell activation
Granulysin*	Polyclonal	141Pr	Effector cell tumor killing
KIR2DS1	1127B	142Nd	NK receptor that interacts with DAP12
PD-1	EH12.2H7	143Nd	Activation and checkpoint inhibition
DAP12	406288	144Nd	Signaling adaptor of NK cell activation
CD19	HIB19	145Nd	B cell marker
CD14	M5E2	146Nd	monocyte and macrophage
CD86	IT2.2	147Sm	costimulation
Granzyme A	CB9	148Nd	Effector cell tumor killing
Granzyme K	GM26E7	149Sm	Effector cell tumor killing
CD26	BA5b	150Nd	Dipeptidyl peptidase/adenosine deaminase
TCR-Vd1	REA173	151Eu	gamma delta T cell receptor
CTLA-4	L3D10	152Sm	Checkpoint inhibition
CD69	FN50	153Eu	Lympocyte activation marker
TCRgd	REA591	154Sm	gamma delta T cell identification
EOMES	WD1928	155Gd	Transcription factor for effector lymphocyte function
CD8b	SIDI8BEE	156Gd	CD8 T cell beta-chain
CD16	3G8	157Gd	NK marker
CD39	A1	158Gd	ATP to Adenosine enzyme
PLZF	R17-809	159Tb	Transcription factor, regulates NK cells
NKp30	P30-15	160Gd	NK cell marker
PSGL1	CHO131	161Dy	Adhesion molecule for tissue infiltration
CD56	NCAM16.2	162Dy	Pan-NK cell marker
4-1BB	REA765	163Dy	T cell costimulation
GITR	110416	164Dy	Glucocorticoid-induced receptor - Tregs
NKG2A	131411	165Ho	Effector NK cell marker - negative
CD107a	H4A3	166Er	Degranulation marker
Grz B	GB11	167Er	Effector cell tumor killing
NKp46	REA808	168Er	Activating receptor on NK cells
TCRVa7.2	REA179	169Tm	Invariant NK T cells
PD-L1	29E.2A3	170Er	PD-1 ligand for checkpoint inhibition
TCR-Vd2	123R3	171Yb	gamma delta T cell receptor
NKG2D	149810	172Yb	Effector NK cell marker - positive
TCRab	T10B9.1A-31	173Yb	alpha-beta T cell receptor
Ki67	8D5	174Yb	Recently proliferated cell marker
Tbet	4B10	175Lu	Transcription factor for NK and effector lymphocyte subets
Perforin	dG9	176Yb	Effector cell tumor killing
CD57	REA769	194Pt	T cell exhaustion marker
HLA-DR	L243	195Pt	Hiscompatability
SLAMF6	292811	196Pt	Trigger cytolytic activity of NK and NKT cells
CD38	HIT2	198Pt	NK adhesion to endothelium, cADP ribose hydrolase
TIGIT	A15153G	209Bi	Inhibitory receptor on Tregs

* Highlighted markers are stained for intracellular detection

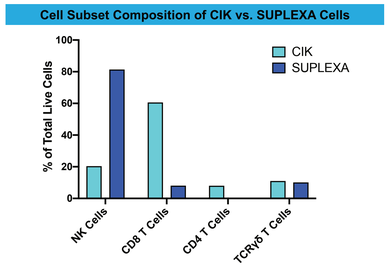
CyTOF Data Analysis Workflow



Cell Subset Overlay opt-SNE Plots Comparing CIK and SUPLEXA Cells



Cell Subset Composition of CIK vs. SUPLEXA Cells



Results

Figure 3: SUPLEXA Cell Phenotype By CyTOF. CyTOF data from SUPLEXA were analyzed to compare functional phenotypes by marker expression profiles.

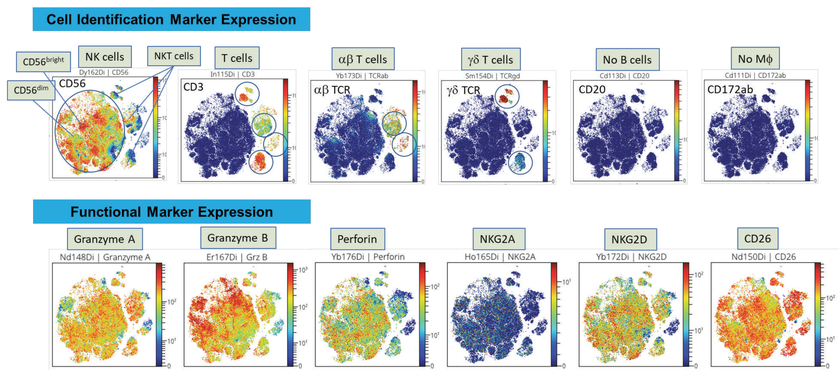
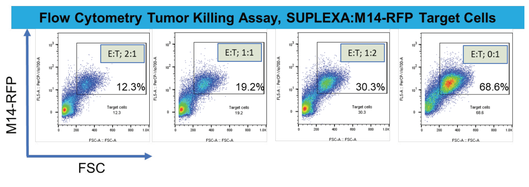


Figure 4: Tumor Cell Cytolysis Activity. SUPLEXA and CIK cells were compared for tumor cell cytolytic activity at low effector to target (M14 melanoma) ratios. Figures show representative killing of M14-RFP target cells as measured by flow cytometry and comparison of tumor killing activity between SUPLEXA and CIK cells. ***SUPLEXA cells are greater than 2-fold more effective at killing tumor cells than control CIK cells.***



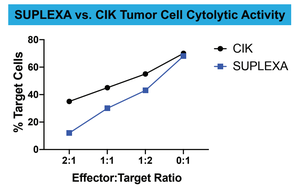
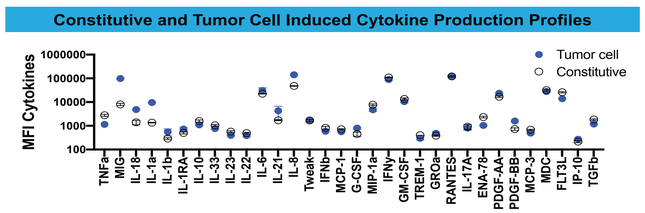


Figure 5: Constitutive and Tumor Target Induced Cytokine Production by SUPLEXA. SUPLEXA cells were incubated without or with M14 melanoma cells for 48 hours and supernatants were tested for cytokines by Luminex. ***SUPLEXA cells produced both constitutive and tumor cell induced cytokines.***



Conclusions

1. Activation of PBMCs by ENLIST cells induces development of potent tumor killing SUPLEXA cells, comprised of known anti-tumor effector cells.
2. SUPLEXA cells are phenotypically and functionally differentiated from control CIK cells; demonstrating the importance of ENLIST cell training.
3. CyTOF analysis of SUPLEXA cells demonstrates unique development of NK cells, NKT-like cells, and CD8 T cells with high expression of tumor killing granzymes and activation markers, *e.g.* NKG2D+, NKG2A-, and CD26.