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CHARACTERIZATION OF A NOVEL AUTOLOGOUS PAN-CANCER CELLULAR IMMUNOTHERAPY

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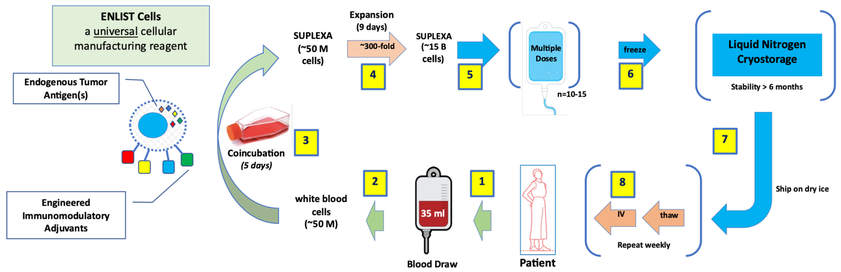
Background

A unique autologous cellular therapeutic (SUPLEXA) has been developed from human PBMC. It is comprised of NK cells, γδ T cells and CD8+ T effector cells, capable of broadly lysing a variety of tumor cell lines in vitro. SUPLEXA cells are manufactured using an efficient 2 weeks xeno-free manufacturing procedure employing two proprietary engineered leukocyte stimulator cell lines (ENLIST) that express an array of immunomodulatory proteins. The SUPLEXA cell manufacturing process is highly reproducible and demonstrates low inter-subject variability in cellular composition. SUPLEXA cells are distinguished from many other cellular approaches in that they are derived from autologous PBMC that have only been stimulated with ENLIST cells through naturally occurring receptors without any genetic modification. **Here, we present our *in vitro* manufacturing process for SUPLEXA cells from PBMCs with comprehensive immunophenotyping of SUPLEXA cells.**

The SUPLEXA cells manufacturing process will use peripheral blood mononuclear cells (PBMCs) from cancer patients. PBMCs are 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 to use as an autologous adoptive immunotherapy. A first-in-human clinical trial for this adoptive cellular immunotherapy for cancer is projected to this year.



Methods

ENLIST cells: Engineered SK-MEL2 melanoma cell lines (APX-DC and APX-L) that express curated sets of > 20 different immunomodulatory proteins that are engineered for membrane expression. ENLIST cells were used as a lyophilized cellular induction reagent for SUPLEXA.

SUPLEXA: PBMCs from normal healthy volunteers 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.

Mass Cytometry (CyTOF): SUPLEXA cells were comprehensively characterized by mass cytometry (CyTOF) using a 47-marker antibody panel. CyTOF data analysis was done using OMIQ for dimensional reduction by opt-SNE and cell subset phenotyping.

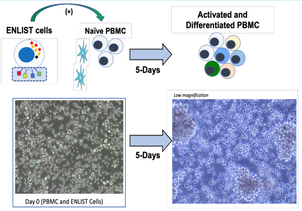
Tumor Cell Killing Assay: Tumor cytolytic activity was measured by flow cytometry using fluorescent tumor cell targets at 2:1, 1:1, and 1:2 effector:target cell ratios.

Cytokines: A 33 cytokine Luminex panel was used to assess cytokine levels in tumor cell 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.

ENLIST Cell Induction of SUPLEXA from PBMCs

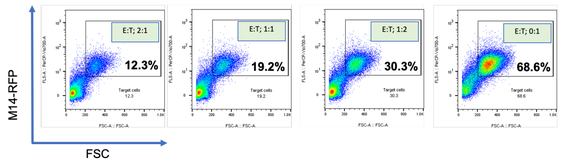


SUPLEXA Cells Develop Broad Tumor Cell Killing Activity

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%

Figure 2: Tumor Cell Cytolysis Assay and Killing Activity. SUPLEXA cells were compared for tumor cell cytolytic activity against fluorescent tumor target cells using a flow cytometry method. Figures show representative killing of M14-RFP target cells as measured by flow cytometry and tumor cytolysis activity against different tumor cell lines (M14, K562, PC3, COLO205).

Flow Cytometry Tumor Killing Assay; SUPLEXA:M14-RFP Targets



Comparison of SUPLEXA Tumor Cell Line Killing Activity

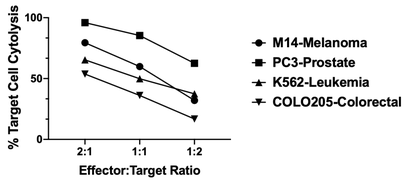
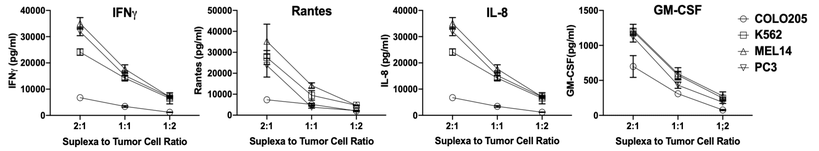


Figure 3: Cytokine Production by SUPLEXA During Tumor Cytolysis. SUPLEXA cells were incubated without or with M14, K562, PC3, or COLO205 cells for 48 hours. Supernatants were tested for cytokines by 33-plex Luminex. Plots of tumor-induced cytokine production by SUPLEXA are shown.

Cytokine Production by SUPLEXA Corresponds to Cytolysis Activity



Results

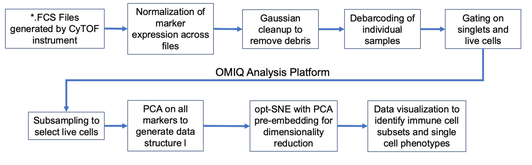
Figure 4: SUPLEXA Cell Phenotyping By CyTOF – Antibody Panel and Data Analysis Workflow. SUPLEXA cells were analyzed to compare single-cell phenotypes using a customized 48-marker CyTOF antibody panel to identify NK cell and T cell subsets as well as transcription factors, adhesion markers, and functional molecules. CyTOF antibody panels, CyTOF data analysis workflow, and a cell subset overlay plot is shown here.

CyTOF Antibody Panel for SUPLEXA Characterization

Marker	Antibody Clone	Metal	Role
CD45	H30	89Y	Pan-leukocyte marker
CD172ab	SE5A5	131Cd	Myeloid cell marker
CD8a	RPA-T8	112Cd	CD8 T cell alpha-chain
CD20	2H7	113In	B cell marker
CD4	RPA-T4	116Cd	CD4 T cell marker
CD3	UCHT1	110In	All T cell marker
DNAM1	DX11	156Cd	Adhesion molecule for NK cell activation
Granulysin*	Polyclonal	141Pr	Effector cell tumor killing
KIR2DS1	11Z7B	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 inhibitor
CD69	FN50	153Eu	Lymocyte activation marker
TCRgd	REA591	154Sm	gamma delta T cell identification
ICOS-L	WO1928	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
PDL1	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 subsets
Perforin	dG9	176Yb	Effector cell tumor killing
CD57	REA769	194Pt	T cell exhaustion marker
HLA-DR	L243	195Pt	Histocompatability
SLAMF6	292811	196Pt	Trigger cytolytic activity of NK and NKT cells
CD58	HT2	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 Plot

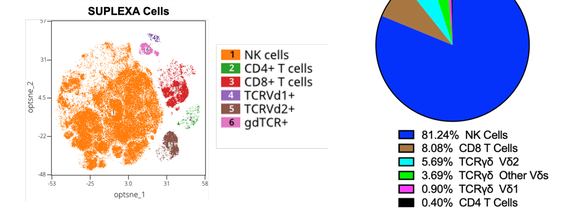
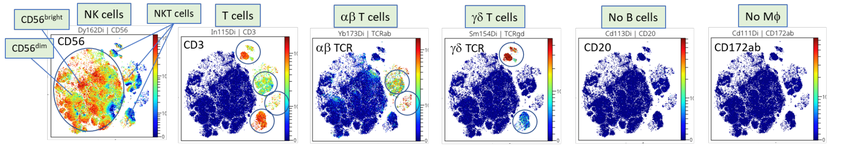
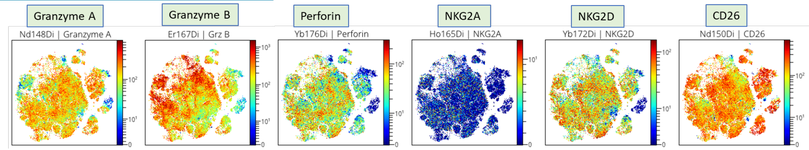


Figure 5: SUPLEXA Cell Phenotyping By CyTOF. CyTOF data from SUPLEXA were analyzed to compare functional phenotypes by marker expression profiles.

Cell Identification Marker Expression



Functional Marker Expression



Conclusions

1. The manufacture of SUPLEXA cells from PBMCs generates immune effector cells with potent cytolysis activity against multiple tumor cell line targets.
2. Comprehensive phenotyping of SUPLEXA cells by CyTOF indicates that SUPLEXA cells are a mixture of NK cells, CD8+ T cells, CD56+ NK-like T cells, and γδ T cells that express activation phenotypes (NKG2D, CD26) and high-levels of killer cell factors (granzymes and perforin).
3. SUPLEXA cells are a novel autologous cellular therapeutic for cancer.