Engineered immunostimulatory cells can convert PBMCs from chronic lymphocytic leukemia (CLL) patients into potent tumor killing immune cells

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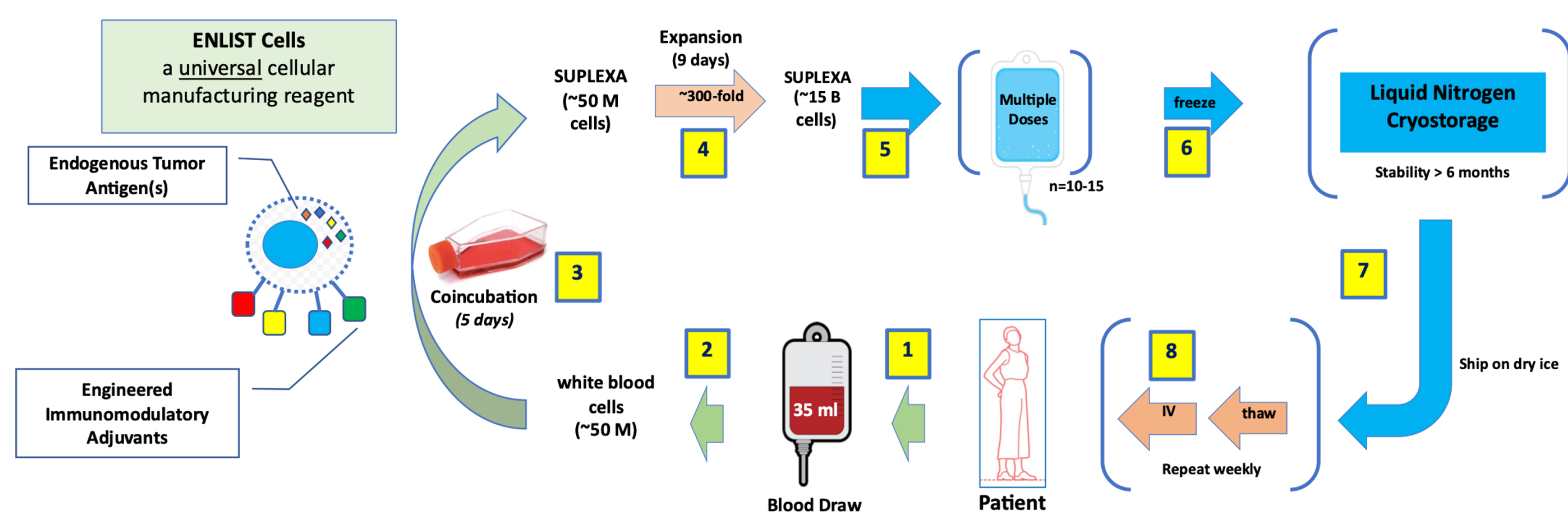
ABSTRACT #7517

Background

A unique autologous cellular therapeutic (**SUPLEXA**) has been developed from human PBMC. It is comprised of NK cells, NKT-like 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. This process leads to a 300-fold expansion of NK cells, CD8+ T cells, NKT-like cells, and TCRγδ T cells that are called SUPLEXA cells, which will be cryopreserved, and then transferred back into patients as an autologous immune cell therapy for cancer. **In this study, PBMCs from CLL patients were used to generate SUPLEXA cells as a first approach to comparatively profile SUPLEXA cells from cancer patients and normal healthy volunteers (NHVs).**

The SUPLEXA cell manufacturing process uses 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 novel adoptive cellular immunotherapy for cancer is projected to begin later this year.



Methods

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

SUPLEXA cell generation: Two million (M) PBMCs isolated from direct blood draws from 10 CLL patients or 5 NHVs were incubated with 0.4 M freeze/thaw killed ENLIST cells for 5 days in XVIVO-15 medium with 2% heat-inactivated human AB serum (XAB2) and then split 1:15 in XAB2 containing IL-7 and IL-15 to expand. 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 an analysis workflow of dimensional reduction by PCA embedded opt-SNE using OMIQ

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. Cytolysis was measured at 48 hours.

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

CyTOF Antibody Panel

Marker	Metal	Role
CD45	89Y	Pan-leukocyte marker
CD172ab	111Cd	Myeoloid cell marker
CD8a	112Cd	CD8 T cell alpha-chain
CD20	113In	B cell marker
CD4	114Cd	CD4 T cell marker
CD3	115In	All T cell marker
DNAM1	116Cd	Adhesion molecule for NK cell activation
Granulysin*	141Pr	Effector cell tumor killing
KIR2DS1	142Nd	NK receptor that interacts with DAP12
PD-1	143Nd	Activation and checkpoint inhibition
DAP12	144Nd	Signaling adaptor of NK cell activation
CD19	145Nd	B cell marker
CD14	146Nd	monocyte and macrophage
CD86	147Sm	costimulation
Granzyme A	148Nd	Effector cell tumor killing
Granzyme K	149Sm	Effector cell tumor killing
CD26	150Nd	Dipeptidyl peptidase
TCR-Vd1	151Eu	gamma delta T cell receptor
CTLA-4	152Sm	Checkpoint inhibition
CD69	153Eu	Lympocyte activation marker
TCRgd	154Sm	gamma delta T cell identification
EOMES	155Gd	TF for effector lymphocyte function
CD8b	156Gd	CD8 T cell beta-chain
CD16	157Gd	NK marker
CD39	158Gd	ATP to Adenosine enzyme
PLZF	159Tb	Transcription factor, regulates NK cells
NKp30	160Gd	NK cell marker
PSGL1	161Dy	Adhesion molecule for tissue infiltration
CD56	162Dy	Pan-NK cell marker
4-1BB	163Dy	T cell costimulation
GITR	164Dy	Glucocorticoid-induced recetptor - Tregs
NKG2A	165Ho	Effector NK cell marker - negative
CD107a	166Er	Degranulation marker
Grz B	167Er	Effector cell tumor killing
NKp46	168Er	Activating receptor on NK cells
TCRVa7.2	169Tm	Invariant NK T cells
PD-L1	170Er	PD-1 ligand for checkpoint inhibition
TCR-Vd2	171Yb	gamma delta T cell receptor
NKG2D	172Yb	Effector NK cell marker - positive
TCRab	173Yb	alpha-beta T cell receptor
Ki67	174Yb	Recently proliferated cell marker
Tbet	175Lu	TF for NK and effector lymphocyte subets
Perforin	176Yb	Effector cell tumor killing
CD57	194Pt	T cell exhaustion marker
HLA-DR	195Pt	Hiscompatability
SLAMF6	196Pt	Trigger cytolytic activity of NK and NKT cells
CD38	198Pt	NK adhesion to endothelium
TIGIT	209Bi	Inhibitory receptor on Tregs

* Highlighted markers are stained for intracellular detection

Results

Figure 1: SUPLEXA Cell Manufacturing and Study Design. 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 to illustrate the strong immune reaction induced by ENLIST cells**

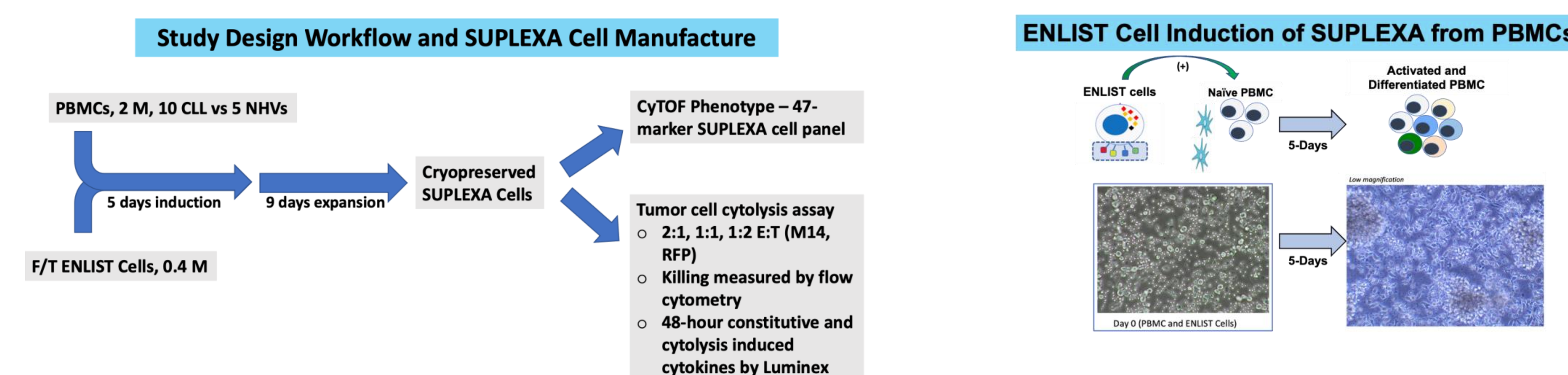
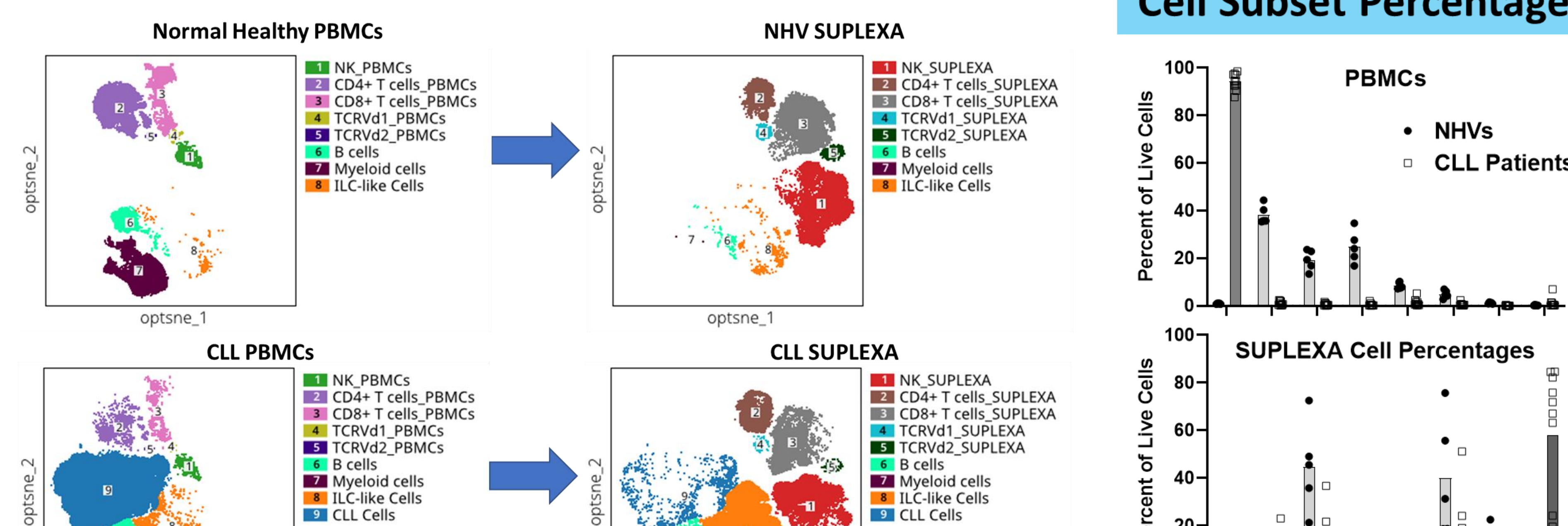


Figure 2: SUPLEXA Cell Phenotyping By CyTOF: SUPLEXA cells from CLL patients and NHVs were analyzed to compare single-cell phenotypes using a customized 47-marker CyTOF antibody panel to identify NK cell and T cell subsets as well as transcription factors, adhesion markers, and functional molecules. Our CyTOF data analysis workflow, cell subset overlay plots of SUPLEXA differentiation of PBMCs from NHVs vs. CLL patients, and cell marker expression profiles of SUPLEXA from NHVs and CLL patients for comparison are shown. **Results indicate that SUPLEXA cells can be generated from CLL PBMCs that contain 90% CLL cells.**



Cell Subset Percentages

PBMCs: NHVs, CLL Patients

SUPLEXA Cell Percentages

Percent of Live Cells

CLL, CD4+ T, CD8+ T, Myeloid, B Cells, NK Cells, γδ T, ILC-like

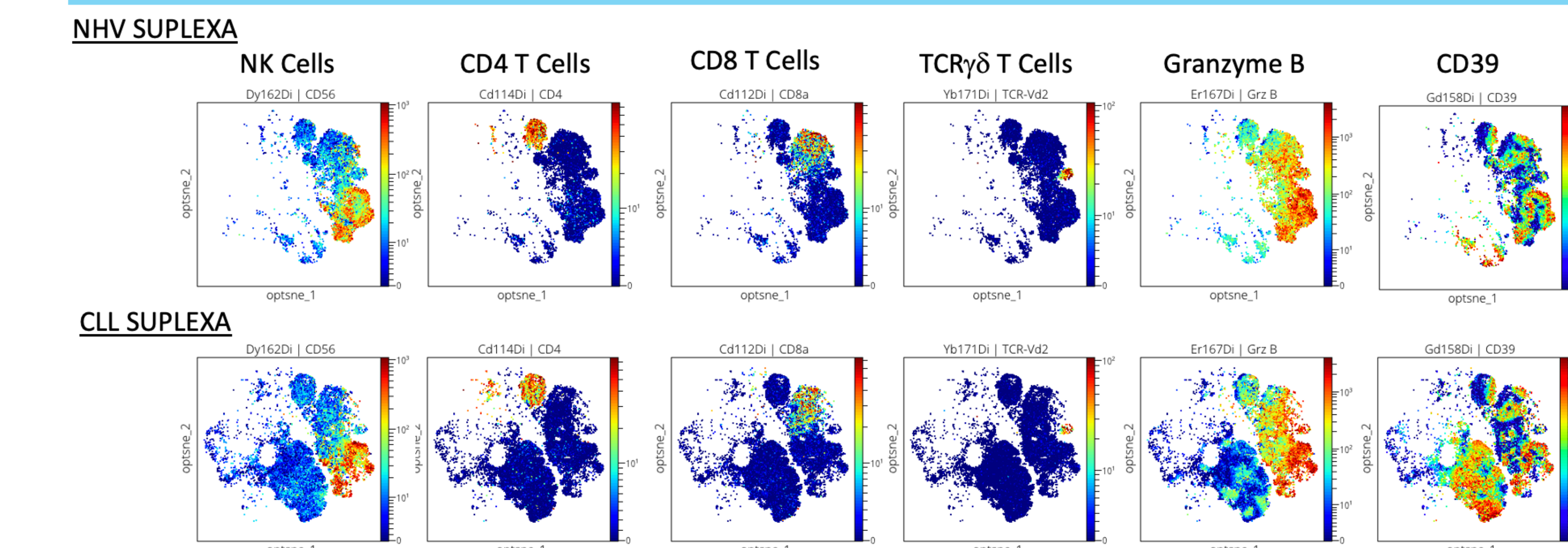
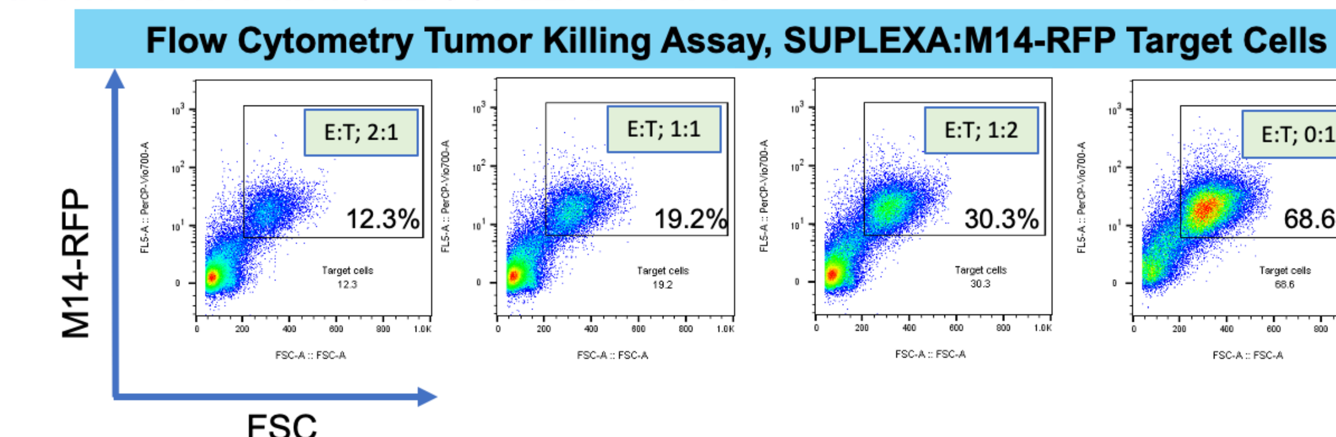


Figure 3: Tumor Cell Cytolysis Assay and Killing Activity. SUPLEXA cells from NHVs and CLL patients were compared for tumor cell cytolytic activity against fluorescent tumor target cells using a flow cytometry method. Figures show representative killing of M14-RFP target cells and comparative tumor cytolysis activity SUPLEXA from 2 NHVs and 10 CLL patients. **Results show potent and similar tumor cytolytic activity between NHV and CLL SUPLEXA cells.**



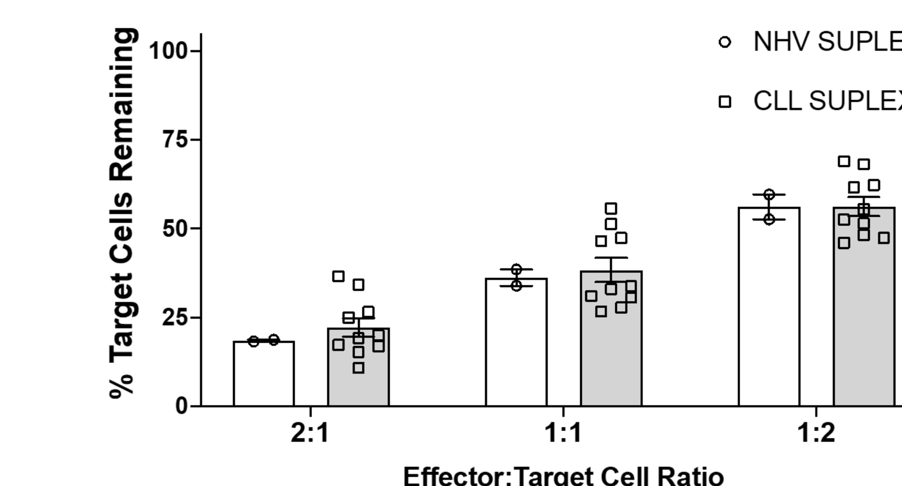
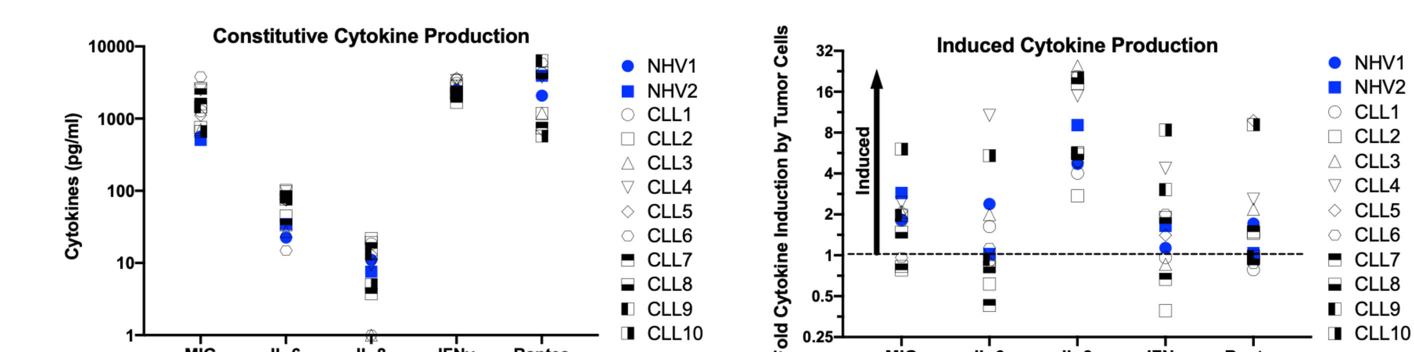


Figure 4: Cytokine Production by SUPLEXA During Tumor Cytolysis. SUPLEXA cells were incubated without or with M14 tumor cells for 48 hours. Supernatants were tested for cytokines by 33-plex Luminex. Plots of constitutive and tumor cell (M14) induced cytokine production by SUPLEXA from NHVs and CLL patient are shown. **Results show comparable levels of cytokine production between CLL and NHV SUPLEXA cells.**



Conclusions

1. We show consistent ENLIST cell induced generation of SUPLEXA cells from CLL patients and CLL cells die or are killed during the SUPLEXA manufacturing process
2. CyTOF single-cell phenotyping of SUPLEXA cells from CLL patients and NHVs showed overlapping phenotypes, but also some important differences:
   - No TCRγδ T cell expansion from CLL PBMCs
   - Expansion of ILC-like cells in CLL patients – CD45+,CD39+,CD69+, Tbet+, GranzymeB and SLAMF6+ cells without T, B, myeloid, or NK markers
3. SUPLEXA cells from NHV and CLL patients showed identical levels of potent tumor cell killing activity and cytokine production profiles with normal distribution of heterogeneity

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