Poster # 164

Potent Tumor Organoid Infiltration and Killing by PBMC-Derived Effector Cells

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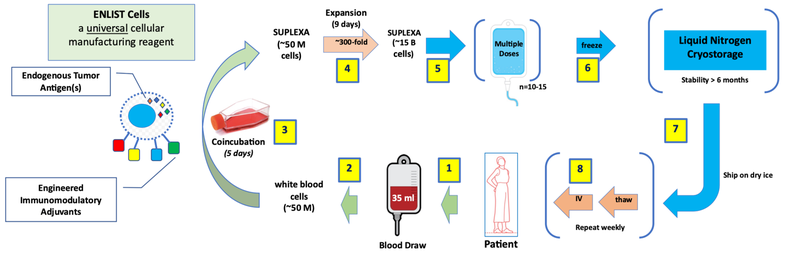
Background

Alloplex Biotherapeutics has developed a novel autologous cellular therapy for cancer 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). The 2-week manufacturing process from PBMCs consistently results 300-fold expansion of NK cells, CD8+ T cells, TCRγδ T cells, NKT cells and some CD4+ T cells, collectively called SUPLEXA therapeutic cells. SUPLEXA cells will be delivered back to cancer patients via intravenous administrations on a weekly schedule as an autologous adoptive cellular immunotherapy for cancer. In this study, we tested SUPLEXA cells developed from normal healthy volunteer PBMCs for their ability to infiltrate and kill patient-derived tumor organoids (PDO) as a pre-clinical assessment for potency against 2 different types of tumor organoids.

The SUPLEXA cells manufacturing process uses peripheral blood mononuclear cells (PBMCs) from cancer patients, which are then stimulated with freeze/thaw non-viable ENLIST cells for a 5-day induction period. This is followed by a 9-day cytokine-induced expansion period. SUPLEXA cells are then cryopreserved until use to as a cellular immunotherapy.

SUPLEXA Cell Manufacture and Autologous Adoptive Cellular Immunotherapeutic Strategy



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.

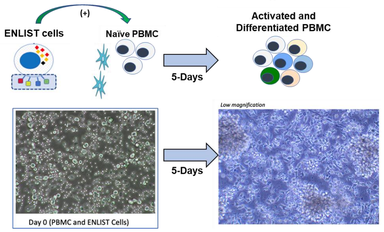
SUPLEXA: PBMCs from normal healthy volunteers were co-incubated with freeze/thaw dead ENLIST cells for 5 days followed by expansion in culture medium containing cytokine support. After 9 days, SUPLEXA cells were harvested and cryopreserved.

Patient-Derived Organoid (PDO) Killing Assays: Tumor organoids derived from colorectal cancer (CRC) or non-small cell lung carcinoma (NSCLC) patients were labeled with cell-trace red dye and plated at equal density in a 96-well plate. After 3 days culture, SUPLEXA cells were thawed (82.8% viable) and labeled with cell-trace violet dye. SUPLEXA cells were added to PDO assays at 1:2 serial diluted numbers ranging from 2 x 10^6 to 7.8 x 10^3 per well. Fluorescent images were captured at 24 hours after adding SUPLEXA cells to PDO models to measure PDO size, tumor infiltration, and PDO killing. PDO assays were performed by Champions Oncology (Hackensack, NJ). Data was analyzed by ANOVA with Tukey correction.

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.

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.



Unmodified Cell Lines Used in Secondary Effector Assay	PBMC co-incubated with *Unmodified SK-MEL-2* (% killing)	PBMC coincubated with *ENLIST Cells* ® (% killing at 48 hours 4:1 E:T)
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: What are SUPLEXA Cells? Phenotyping By CyTOF. CyTOF staining results showing SUPLEXA cell composition and functional phenotypes by marker expression profiles.

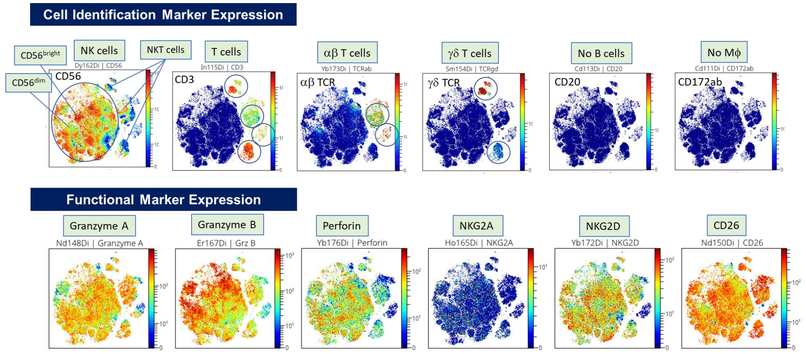
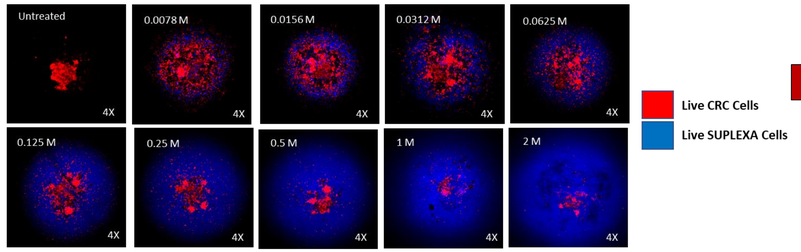


Figure 3: Killing of Colorectal Cancer (CRC) Patient-Derived Organoids (PDO) by SUPLEXA. Fluorescent images showing increased infiltration and killing of fluorescently-labeled (Red) organoids by SUPLEXA cells (Blue). The numbers of SUPLEXA added per well are shown in the panels.



Results

Figure 4: Killing of Non-Small Cell Lung Carcinoma (NSCLC) Patient-Derived Organoids (PDO) by SUPLEXA. Fluorescent images showing increased infiltration and killing of fluorescently-labeled (Red) organoids by SUPLEXA cells (Blue). The numbers of SUPLEXA added per well are shown in the panels.

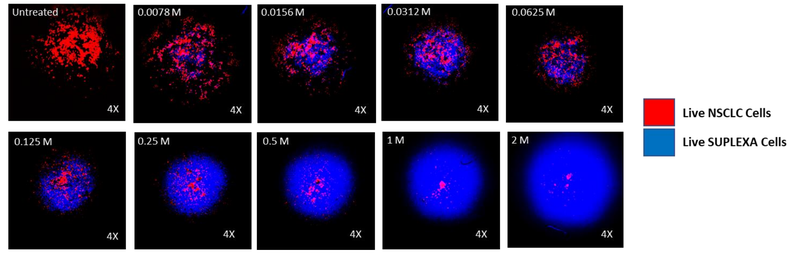
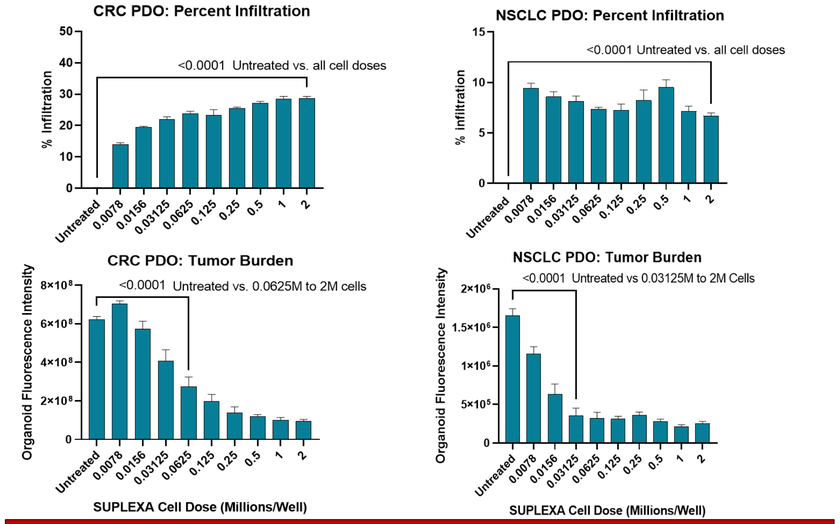


Figure 5: SUPLEXA Cells Infiltrate and Kill Colorectal Carcinoma and Non-Small Cell Lung Carcinoma Organoids. Plots showing the infiltration and killing tumor organoid cells by SUPLEXA cells added at the indicated effector cell doses. Organoids were plated (n=6) and cultured with SUPLEXA at the indicated cell numbers per well.



Conclusions

1. The manufacture of SUPLEXA cells from PBMCs generates a mixture of NK cells, CD8+ T cells, CD56+ NK-like T cells, and γδ T cells with potent patient-derived cancer organoid killing activity.
2. The observed killing of both colorectal and lung cancer organoid models by SUPLEXA cells indicate the potential for broad tumor killing activity.
3. SUPLEXA cells represent a novel autologous cellular therapeutic for cancer that will be tested in phase 1 clinical trial starting by February 2022.