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Alloplex Biotherapeutics
engineering advanced cellular therapies

Interleukin-10 and transforming growth factor-β do not suppress tumor killing activity of PBMC-derived SUPLEXA cells

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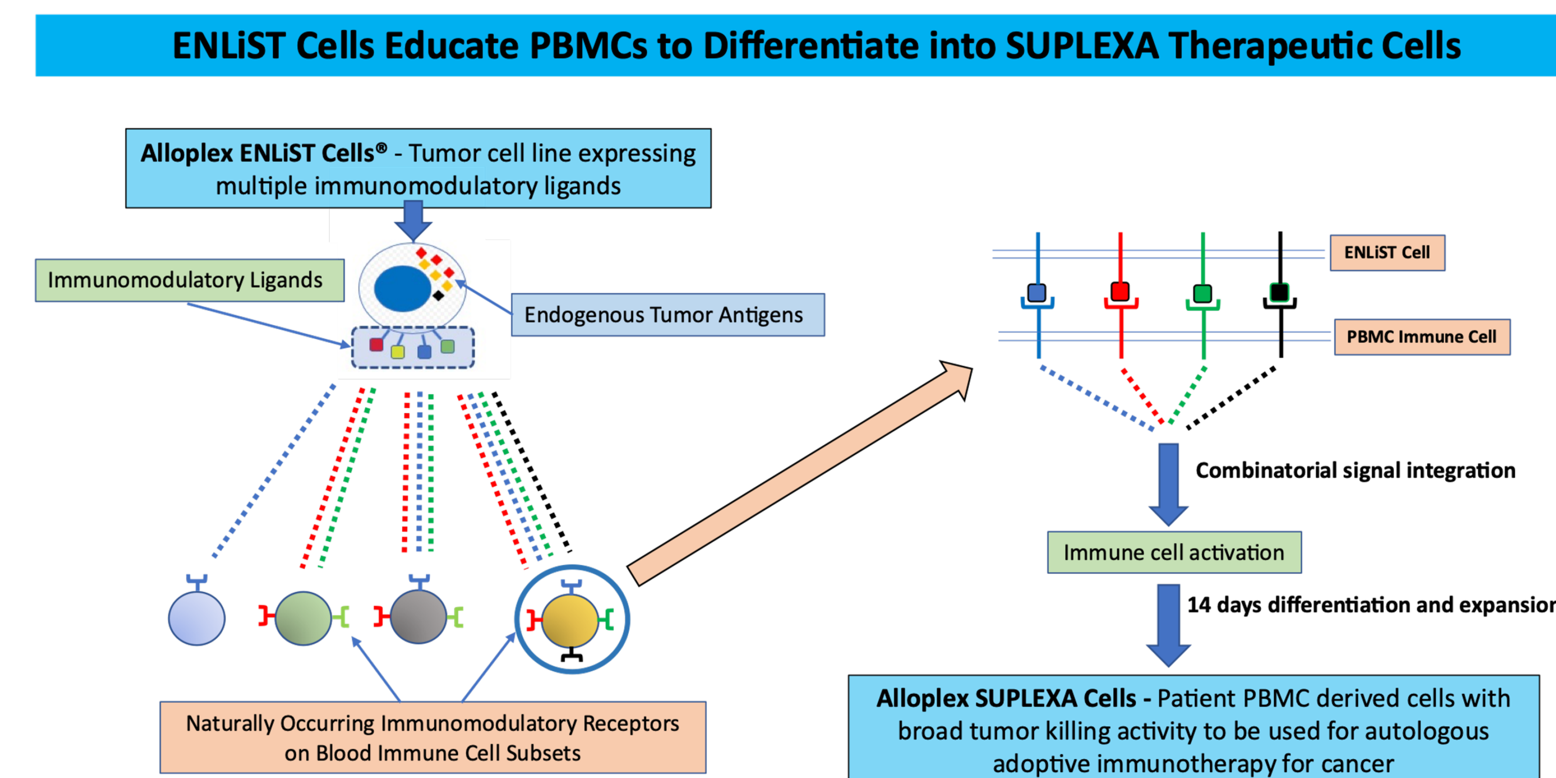
Background

A unique autologous cellular therapeutic called **SUPLEXA cells** has been developed from human PBMC. SUPLEXA cells are a mixture of NK cells, CD8+ T cells, γδ T cells, and NK-like T cells that can broadly kill tumor cell lines *in vitro*. They are manufactured from PBMCs using an efficient 2 weeks xeno-free manufacturing procedure employing two proprietary engineered leukocyte stimulator cell lines call **ENLIST cells** that are melanoma cell lines that express an array of immunomodulatory proteins. SUPLEXA cells are manufactured from peripheral blood mononuclear cells (PBMCs) by stimulation with ENLIST cells for a 5-day induction period, which is then followed by a 9-day cytokine-driven expansion period. The process results in a sufficient numbers of SUPLEXA cells that are then cryopreserved to be used as an autologous adoptive cellular immunotherapy for cancer. The SUPLEXA approach to autologous adoptive immune cell therapeutics is distinguished from many other cellular approaches because they are derived from PBMC that have been stimulated by ENLIST cells through naturally occurring receptors without any genetic modification of the therapeutic cells. **In this study, we assessed the impact of two immunosuppressive cytokines known to be present in the tumor microenvironment (IL-10 and TGF-β) on the tumor cytolytic function and cytokine release of therapeutic SUPLEXA cells.**

ENLiST Cells Educate PBMCs to Differentiate into SUPLEXA Therapeutic Cells



Methods

ENLIST cells: An engineered tumor cell line that express a curated set of immunomodulatory proteins engineered for membrane expression.
Therapeutic SUPLEXA Cells: Peripheral blood mononuclear cells (PBMCs) from normal healthy volunteers were co-incubated with ENLIST cells for 5 days, then expanded for an additional 9 days in culture medium containing cytokine support. After expansion, SUPLEXA cells were harvested and cryopreserved in liquid nitrogen.
Tumor Cell Killing Assay: SUPLEXA cells were thawed, washed in culture media, and used directly in tumor cell cytolysis assays. Tumor cytolytic activity was measured by flow cytometry using fluorescent tumor cell targets at various effector to target cell ratios. Percent target cell cytolysis was calculated as the percentage reduction in fluorescent tumor cells after 48-hours coculture as compared to tumor cells without effector cell addition.
Mass Cytometry (CyTOF): SUPLEXA 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.
Cytokines: A 33 cytokine Luminex panel was used to assess cytokine levels in tumor cell cytolysis supernatants collected at 48 hours.

Results

Figure 1: What are SUPLEXA Cells? SUPLEXA cells for single-cell phenotypes using a customized 48-marker CyTOF antibody panel to identify immune cell lineages with focus on NK cell and T cell subsets as well as transcription factors, adhesion markers, and functional molecules. **SUPLEXA cells are a mixture of NK cells, CD8 T Cells, TCRγδ T cells, NK-like T Cells, and CD4 T Cells.**

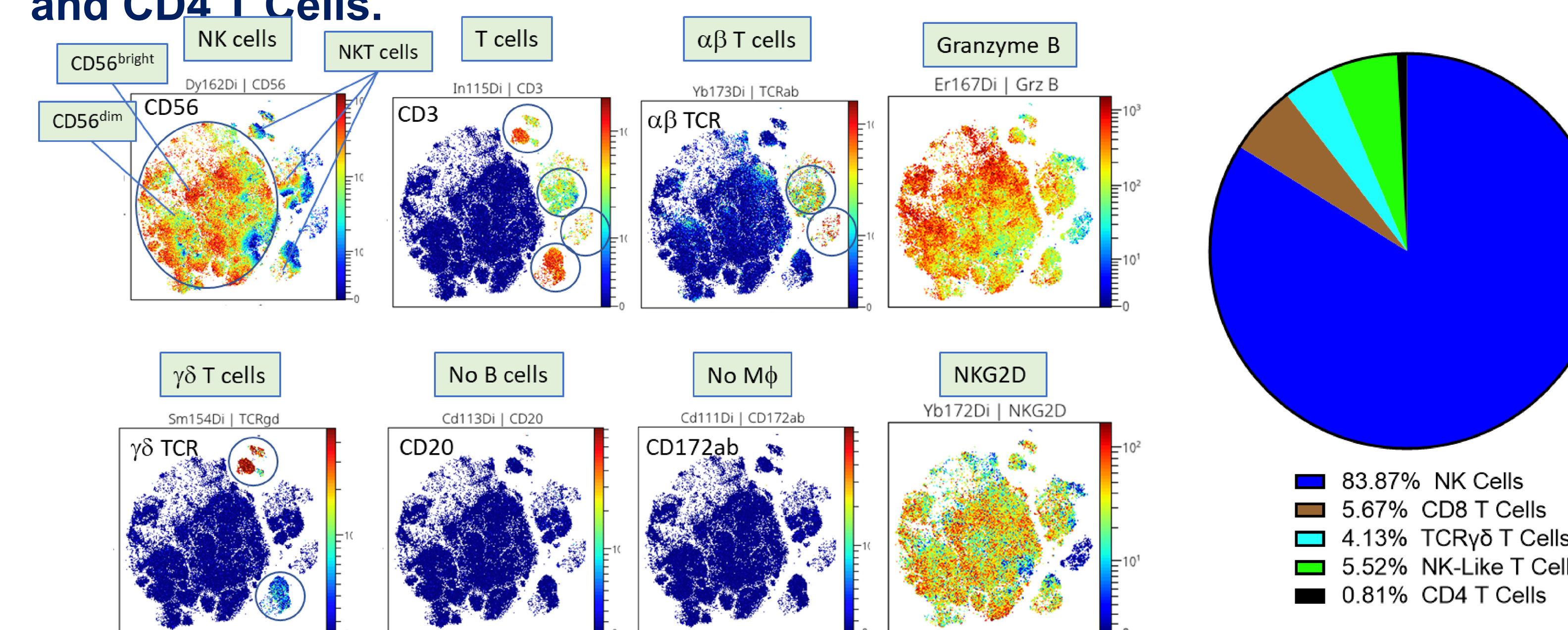


Figure 2: SUPLEXA Cells Acquire Broad and Potent Tumor Cell Cytolysis Activity. SUPLEXA cells were compared for tumor cell cytolytic activity against fluorescent tumor target cells using a flow cytometry method. Figures show representative killing of M14-RFP melanoma target cells as measured by flow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

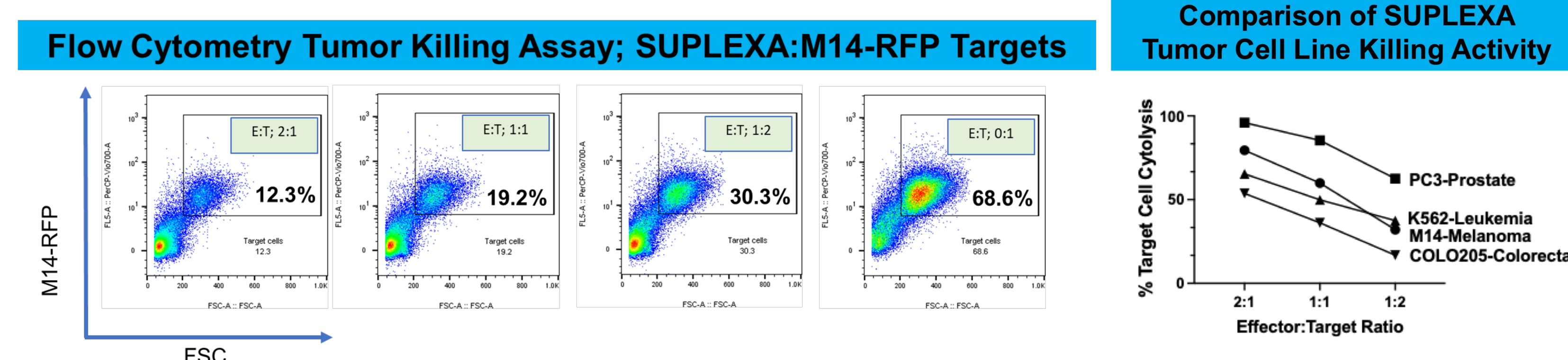
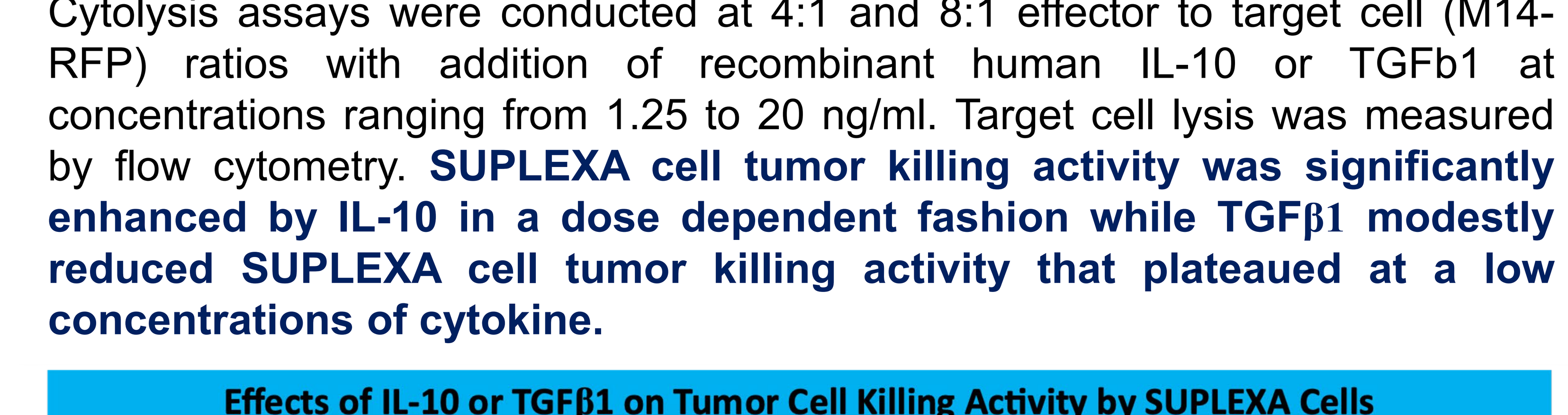


Figure 3: Influence of 2 major immunosuppressive cytokines (IL-10 and TGFβ) on the tumor cell killing activity of SUPLEXA cells. Cytolysis assays were conducted at 4:1 and 8:1 effector to target cell (M14-RFP) ratios with addition of recombinant human IL-10 or TGFb1 at concentrations ranging from 1.25 to 20 ng/ml. Target cell lysis was measured by flow cytometry. **SUPLEXA cell tumor killing activity was significantly enhanced by IL-10 in a dose dependent fashion while TGFβ1 modestly reduced SUPLEXA cell tumor killing activity that plateaued at a low concentrations of cytokine.**

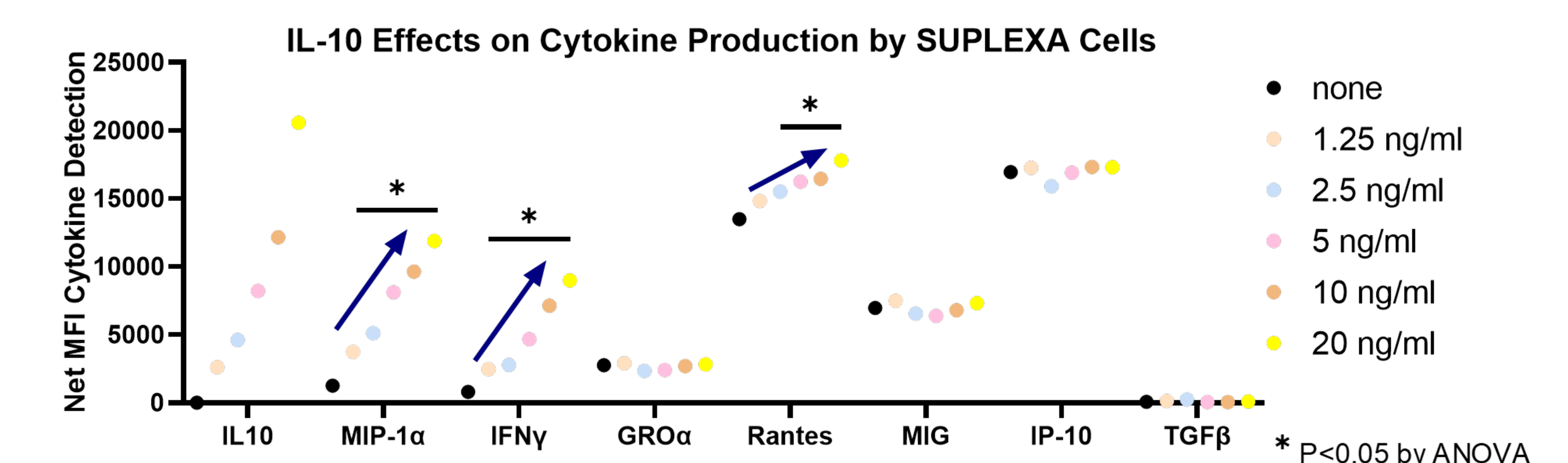
Effects of IL-10 or TGFβ1 on Tumor Cell Killing Activity by SUPLEXA Cells

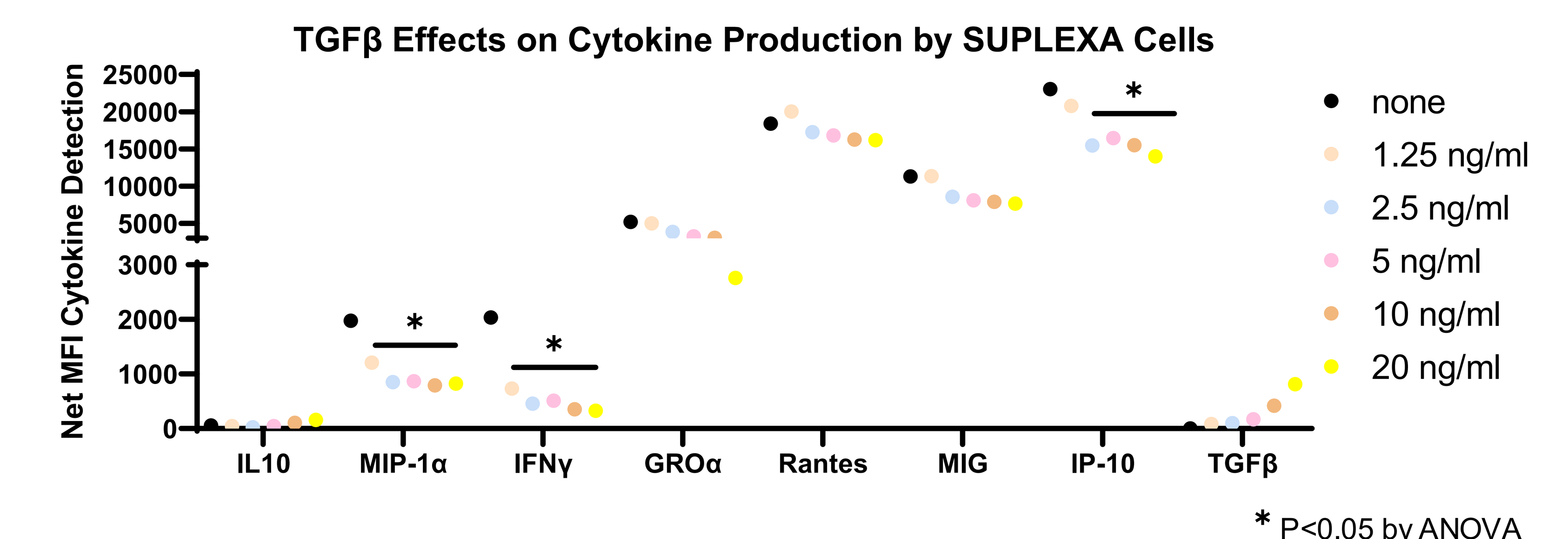


Results

Figure 4: Influence of IL-10 or TGFβ on cytokine production by SUPLEXA cells during tumor cytolysis. 48-hour supernatants from tumor cell cytolysis assays performed at 4:1 effector to tumor target cell ratios were tested for cytokine production profiles by Luminex assays. The effects of human IL-10 or TGFβ1 additions at 1.25 to 20 ng/ml were compared. **Interleukin-10 significantly increased production of MIP-1α and IFNγ, while more modestly raised Rantes levels, all in a dose-dependent manner. In contrast TGFβ1 reduced IFNγ and MIP-1α production, while more modestly reduced IP-10 production.**

IL-10 or TGFβ1 Effects on Cytokine Production by SUPLEXA Cells During Tumor Cell Cytolysis





Conclusions

1. The manufacture of SUPLEXA cells from PBMCs generates immune effector cell types with potent and broad tumor cell killing activity
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, TCRγδ cells, and CD4 T cells that express high-levels of killer cell factors (granzymes and perforin) and activation markers (not shown).
3. IL-10 significantly enhanced the killing activity and production of IFNγ, MIP-1α, and Rantes by SUPLEXA cells in a dose-dependent fashion.
4. TGFβ1 modestly suppressed SUPLEXA cell mediated tumor cell killing activity and reduced IFNγ and MIP-1α production
5. SUPLEXA cells are entering Phase 1 clinical testing in Australia.