Exploratory immune phenotyping of longitudinal blood samples from the first-in-human phase 1 clinical trial of a novel autologous cellular therapy in patients with metastatic solid tumors

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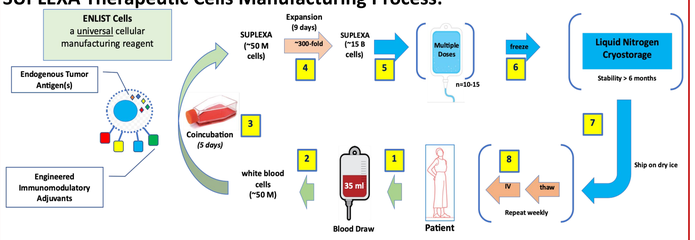
Background and Introduction

- Alloplex Biotherapeutics has developed a novel autologous cellular immunotherapy that is prepared from patient PBMCs called SUPLEXA.
- SUPLEXA is a heterogeneous mixture of innate lymphoid (e.g. NK, NKT cells) and T cell subsets (*e.g.* TCR αβ and γδ T cells) without B cells, Tregs, or myeloid lineage cells.
- SUPLEXA cells have been educated/trained *ex vivo* by melanoma cell lines that express an array of immunomodulatory factors that are known to induce tumor killing effector cell types.
- By this novel approach, SUPLEXA cells acquire potent and broad tumor cell killing activity
- SUPLEXA therapeutic cells are given back to the cancer patient as an autologous cellular immunotherapy.

Methods

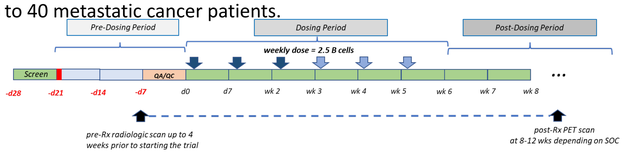
SUPLEXA Therapeutic Cells Manufacturing Process:



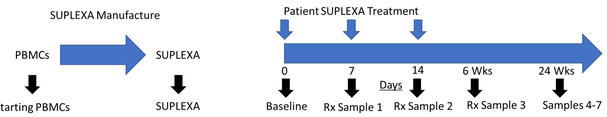
ENLIST Cells: ENLIST cells are an engineered tumor cell line (SK-MEL2) that express a curated set of immunomodulatory proteins engineered for membrane expression.

Therapeutic SUPLEXA Cells: Under GMP manufacturing conditions, peripheral blood mononuclear cells (PBMCs) from this phase 1 clinical trial were cultured with dead (freeze-thawed 5x) ENLIST cells for 8 days, then expanded for an additional 9 days in clinical grade culture medium containing cytokine support. After expansion, SUPLEXA cells were harvested, tested for cytolysis activity and sterility, and cryopreserved in bags for storage in liquid nitrogen.

Clinical Trial Design: SUPLEXA single agent, multiple dose, basket design trial in up to 40 metastatic cancer patients.



Patient Blood Sampling Strategy for Exploratory Immunophenotyping of PBMCs by CyTOF and Plasma by Luminex:



Mass Cytometry (CyTOF): PBMCs and SUPLEXA cells will be comprehensively phenotyped by mass cytometry (CyTOF) using 3 different 48-marker antibody panels to that focus on T cells, innate lymphoid cells, and myeloid cell types. CyTOF data analysis will be done by clustering and dimensional reduction computational approaches using OMIQ

Cytokines: A 40 cytokine Luminex panel was used to assess cytokine levels in patient plasma samples at each time point

Blood immunophenotyping of cancer patients treated with SUPLEXA, a patient-derived autologous cellular immunotherapy, indicates shifts in immune phenotype consistent with improved immune health

Alloplex Biotherapeutics

Brigham and Women's Hospital
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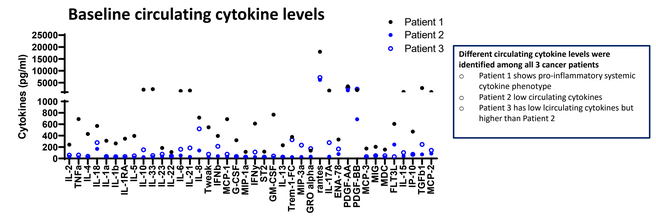
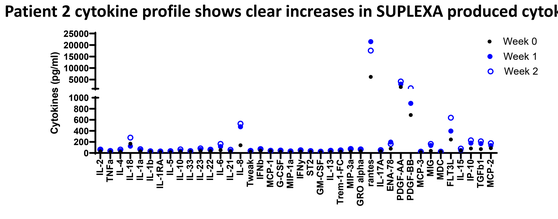
FIGURES AND TABLES

Figure 1: Plasma Cytokine Profiles from the First 3 Enrolled Patients



Patient 2 cytokine profile shows clear increases in SUPLEXA produced cytokines



Patient 1 shows progressive reduction in pro-inflammatory cytokines

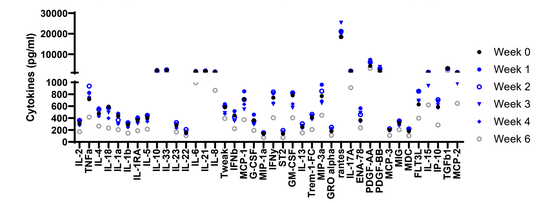


Figure 2: Comprehensive immune cell profiling of SUPLEXA and PBMCs by CyTOF

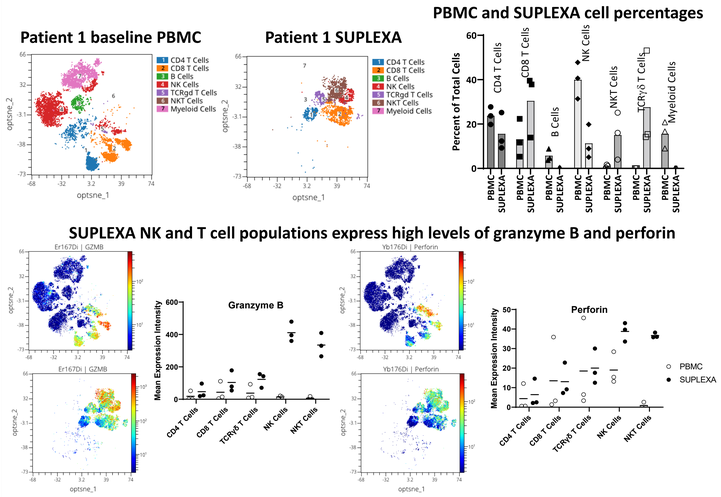
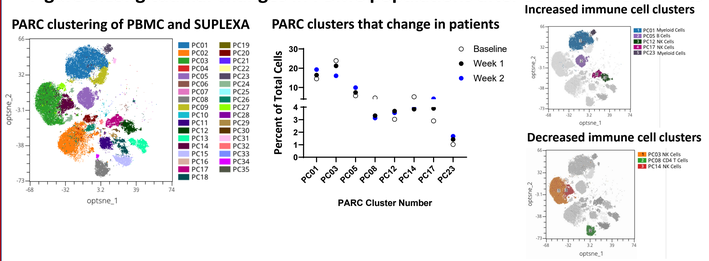


Figure 3:Longitudinal changes in PBMC populations after SUPLEXA treatments



Results and Conclusions

1. Circulating cytokine levels showed modulation of systemic cytokine levels in all 3 patients after SUPLEXA treatments
2. SUPLEXA cells showed highly similar phenotypes between all 3 patients showing induction of the predicted SUPLEXA cell populations – NK and NKT cells, CD8 and CD4 T cells, TCRγδ T cells, with no B cells or myeloid cells.
3. T cells, NK cells, and NKT cells in SUPLEXA showed high expression of the cytotoxic effector enzymes, granzyme B and perforin.
4. Preliminary analysis of longitudinal PBMCs by CyTOF suggest that several specific subpopulations of myeloid cells, NK cells, CD4 T cells, and B cells were affected by SUPLEXA treatment suggesting a change in immune health