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 xenofree 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 adaptive 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 OMIGA 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 cytolytic supernatants.

Results
Figure 1: SUPLEXA Cell Manufacturing. SUPLEXA 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.

Figure 2: Tumor Cell Cytolysis Assay and Killing Activity. SUPLEXA cells were compared for tumor 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 cytolytic activity against different tumor cell lines (M14, K562, PC3, COLO205).

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.

Conclusions
1. The manufacture of SUPLEXA cells from PBMCs generates immune effector cells with potent cytokolytic 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, CD28) and high levels of killer cell factors (granzymes and perforin).
3. SUPLEXA cells are a novel autologous cellular therapeutic for cancer.

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