ENLIST-mediated training of PBMCs from AML patients yields activated cells with anti-tumor function

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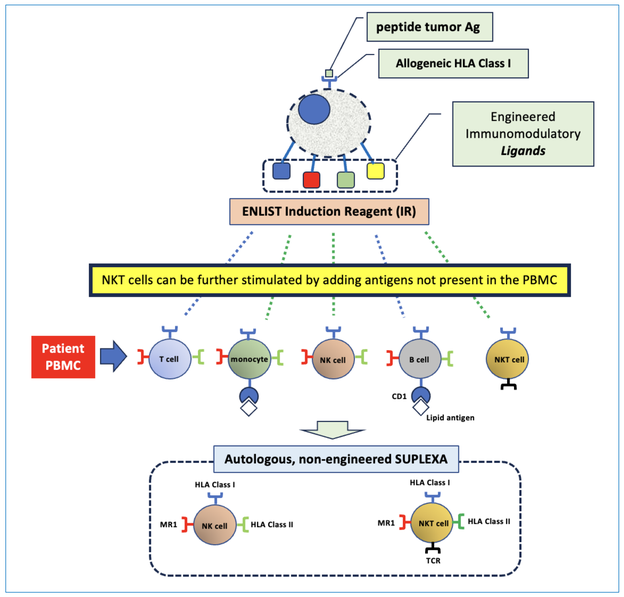
Background

SUPLEXA therapeutic cells are an autologous, non-engineered cell therapy derived from patient PBMCs isolated from 50 mL of whole blood. The manufacturing process is mediated by coincubation of the PBMC with the ENLIST induction reagent, a proprietary cell line expressing an array membrane bound immunomodulators. SUPLEXA cells are composed of highly activated NK and type II NKT cells and are broadly cytolytic against an array of tumor cell lines *in vitro* without harming normal cells. SUPLEXA cells express features that enable direct tumor lysis as well as characteristics of antigen presenting cells ('APCs') such as HLA Class II and T cell costimulator CD86. Phase 1 clinical findings in an open-label, single-agent survey were overwhelmingly positive showing pristine safety and clinical efficacy in end-stage cancer patients with durable responses in colorectal, kidney, and melanoma patients.

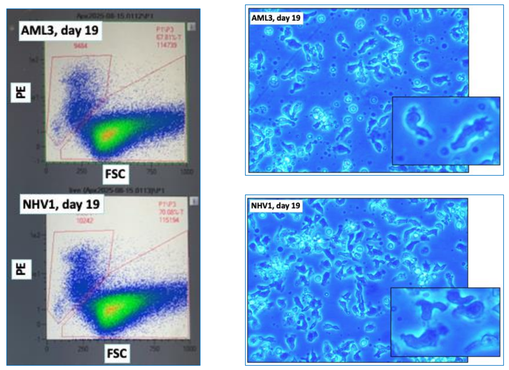
Here, we applied our ENLIST immune cell training platform to PBMC from AML patients to test whether the resulting SUPLEXA cells were comparable to those made from normal healthy volunteers. We demonstrate that SUPLEXA cells manufactured from AML PBMCs show similar anti-tumor features as those from normal healthy volunteer (NHV) PBMCs. This suggests that our ENLIST platform could be used to develop an adoptive cellular immunotherapy to treat AML patients.

SUPLEXA manufacturing



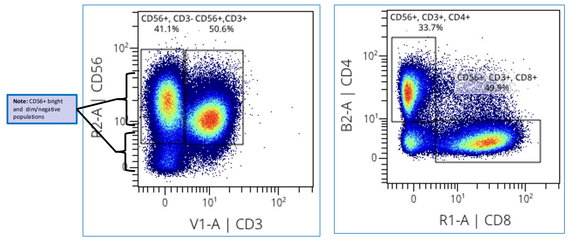
SUPLEXA manufacturing involves the coincubation of PBMC with ENLIST; a tumor cell line modified to express a proprietary combination of membrane expressed immunomodulatory factors. SUPLEXA cells are induced and expand within a 21-day culture period. The system has been optimized for maximal proliferation, differentiation, cytokine production and induction of anti-tumor cytolytic function.

Morphology



Clusters form by Day 5 of SUPLEXA induction. Flow cytometry micrographs of fully formed SUPLEXA cells at day 19 of culture. AML and NHV have a similar activated FSC/SSC flow and cell morphology.

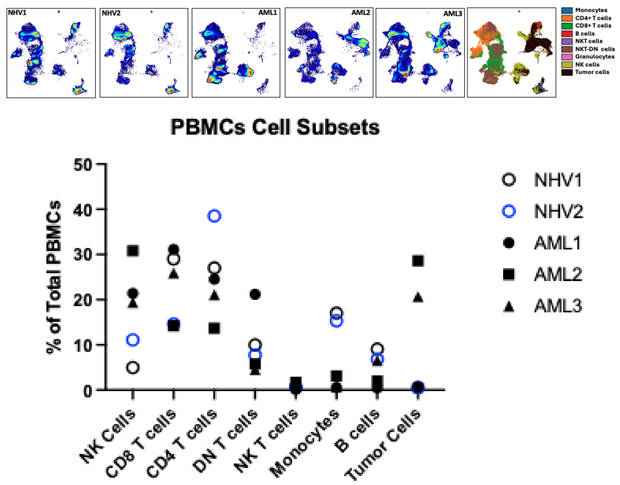
SUPLEXA is comprised of NK and NKT cells



	CD3-	CD3+	CD4+	CD8+	CD4-/CD8-
CD56-	~8%	0%	+++++	+++++	++
CD56+	41%	51%	34%	53%	16%

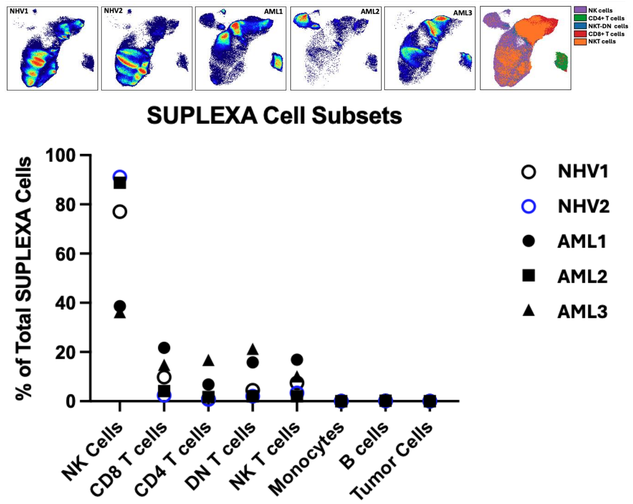
Flow cytometry analysis of SUPLEXA cells showing major lineages. All SUPLEXA cell preparations show a similar phenotype. SUPLEXA preparations are comprised entirely of lymphoid cells and do not contain cells of the myeloid lineage, B cells, or residual tumor cells.

CyTOF of starting PBMC samples



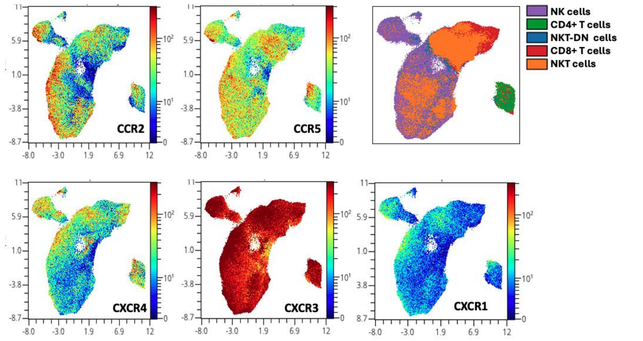
CyTOF analysis of starting PBMC samples from NHV and AML patients with tabulation of major lineages. AML starting PBMC samples contain more NK cells, fewer monocytes and variable numbers of **tumor cells.**

CyTOF of resulting SUPLEXA cells



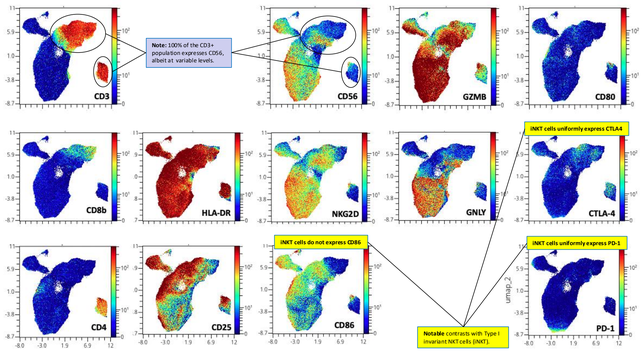
CyTOF analysis of the resulting SUPLEXA cells and tabulation of major lineages. All SUPLEXA cell preparations show similar immune cell types.

Distribution of chemokine receptor for SUPLEXA cells



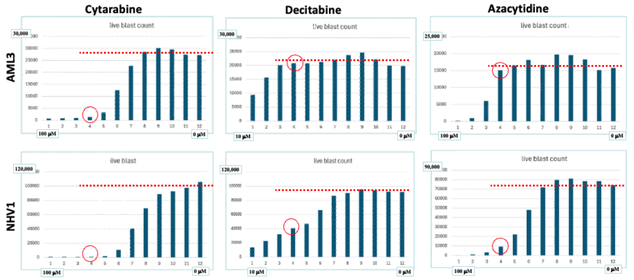
CyTOF analysis of chemokine receptor distribution in SUPLEXA cell preparations. CXCR4 mediates cell migration to bone marrow and CXCR3 mediates migration to the lymphatics.

Distribution of key activation markers for SUPLEXA cells



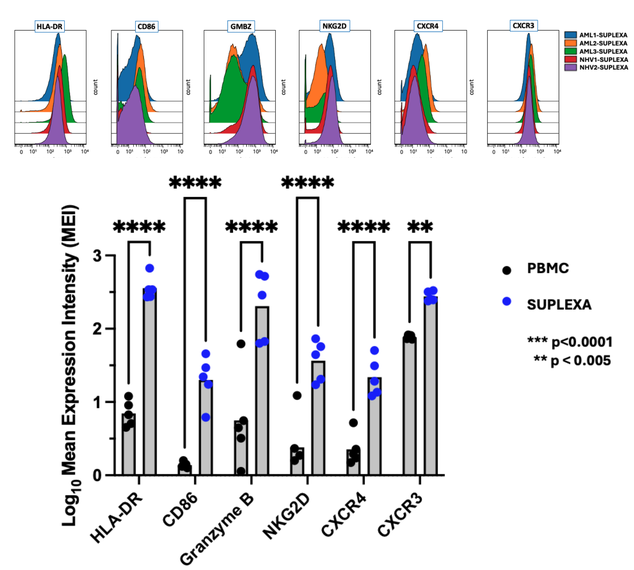
CyTOF analysis of the resulting SUPLEXA cell marker distribution. SUPLEXA NKT cells are CD3, HLA-DR, CD25 and CD86 positive, as well as both CD4 and CD8 single positive or double negative. SUPLEXA NK cells are all CD56, CD25, CD86, NKG2D, HLA-DR, and Granzyme B. NK cells are also positive for Perforin (not shown). SUPLEXA cells are negative for CD80, CTLA4, and PD-1 expression.

AML and NHV SUPLEXA cells are compatible with the standard hypomethylating agents



Semi-log dilution series starting at 10 uM for Decitabine and 100uM for both Cytarabine and Azacytidine. The last dilution contains no drug. **Red circle** indicates expected C_{max} at commonly used clinical doses. Without drug, AML3 SUPLEXA proliferate at a maximum rate of about 25% of NHV #5 over the 72-hour assay period but they are also far less susceptible to HMA inhibition; possibly due to prior exposure and epigenetic adaptation to these HMAs.

Relative levels of activation markers for SUPLEXA cells



CyTOF analysis of activation markers shows AML SUPLEXA preparations are similar to those from NHVs on key activation markers.

Conclusions

Based on prior observations that SUPLEXA cells are cytolytic against a broad array of tumor cells combined with the suggestion from our Phase 1 trial that SUPLEXA cells can migrate to the bone, **we hypothesized that hematologic malignancies could be amenable to adoptive immune cell therapy with SUPLEXA cells.** However, the gating question remained whether SUPLEXA cells could be manufactured from AML PBMCs given the substantial defects in the myeloid lineage and the presence of a substantial number of circulating tumor blasts. Here, we demonstrate that despite the significant defects present in AML PBMC, SUPLEXA cells can readily be made and are substantially similar to those from normal healthy volunteers. Plans are now underway to expand this preliminary study with a wider array of PBMC samples from patients with other hematologic cancers (AML, MDS and MM) at various stages of disease and prior treatment histories. **Based on our findings, translating SUPLEXA cell therapy to these hematologic indications in the context of randomized clinical trials will be possible.**