Poster #378

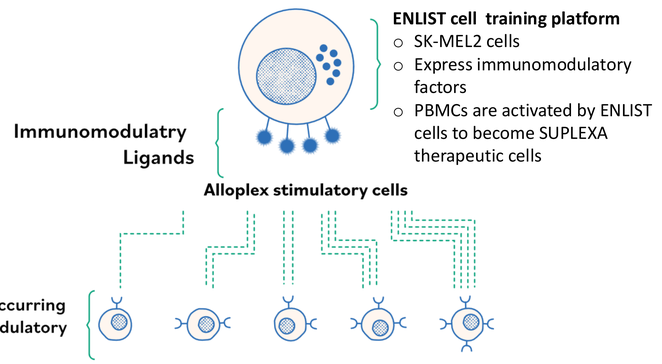
Transcriptional and proteomic insights into the immunomodulatory nature of SUPLEXA cells: An autologous cellular therapy for cancers

[1]J Pulford, [1]E Murzin, [1]D Younger, [2]R Joshi, [3,4]JC Goh, [3]G Kichenadasse, [5]SE Gargosky, [5]F Borriello, and [1,5]J Lederer

[1]Brigham and Women's Hospital/Harvard Medical School, [2]Cancer Research South Australia, [3]Gallipoli Medical Research Foundation, [4]Queensland University of Technology, [4]University of Queensland, [5]Alloplex Biotherapeutics, Inc., Woburn, MA

Background

SUPLEXA cells are PBMC-derived activated white blood cells, comprised predominantly of lymphocytes, notably devoid of B cells, myeloid, cells, and Tregs. SUPLEXA cells are non-engineered, autologous immunotherapeutic cells that are differentiated by an *in vitro* "**training**" process mediated by engineered tumor cells called **ENLIST cells** that express an array of immunomodulatory adjuvants that convert PBMCs into SUPLEXA cells.



SUPLEXA cells are an individualized population of immunotherapeutic cells with the following 4 basic immune properties:

1) **Migratory –** Express chemokine receptors and adhesion molecules.
2) **Cytolytic –** Express high levels of granzymes and perforins.
3) **Antigen Presenting cells –** Express high MHC class II and CD86.
4) **Immunomodulatory –** Modulate peripheral myeloid cell populations.

Study Aims

Specific Aim 1: To profile SUPLEXA cells generated from our Phase 1 clinical trial using CyTOF, RNAseq, and Nanostring technologies.

Specific Aim 2: To perform longitudinal pharmacologic analysis of blood samples from SUPLEXA treated patients. Immune cell subsets in PBMC were analyzed by CyTOF and plasma analytes using Luminex and Olink.

Methods

SUPLEXA and PBMC Mass Cytometry (CyTOF) Analysis. SUPLEXA cells from patients were analyzed with a custom 48-marker CyTOF antibody panel. Cryopreserved PBMCs from SUPLEXA treated patients were stained with two 48-marker CyTOF panels with myeloid or T cell focus. CyTOF data was analyzed by R and OMIQ workflows to deeply profile SUPLEXA phenotypes and longitudinal phenotypic changes in PBMCs from patients.

SUPLEXA Transcriptional Profiling. RNA was prepared from a subset of SUPLEXA cells or PBMCs and subjected to Next Generation RNA sequencing at the MBCF core at the DFCI. RNA was also analyzed by Nanostring technology for further validation of transcriptional profiles. Data was analyzed by Biojupies and STRING platforms to identify significant gene gene transcriptional profiles and networks in therapeutic SUPLEXA cells.

Plasma Cytokine and Biomarker Discovery by Luminex/Olink. Luminex assays for 40 different cytokines were performed on longitudinal patient samples. The Olink Discovery Panel (3,072 proteins) was used on a subset of patient plasma samples at baseline, 1, 2 weeks post treatment as a plasma biomarker discovery approach SUPLEXA treatment.

Phase 1 Clinical Trial Design and Outcomes

Clinical Findings: This poster reports on SUPLEXA-101, 35 patients with histologically or cytologically confirmed measurable solid tumors, radiographically confirmed metastatic cancer who had exhausted standard options. All eligible subjects received a minimum of 3 weekly dose of SUPLEXA of approx. 2.5 billion cells per dose. At the discretion of the investigator, sponsor medical monitor, and in agreement with the subject, additional SUPLEXA infusions were administered when available. Response was assessed by imaging on an 8-12 week schedule.

Therapeutic Responses	N (%)
Overall Response Rate (ORR))	3 (9.4)
Complete Response (CR)	1 (3.1)
Partial Response (PR)	2 (6.3)
Stable Disease (SD)	19 (59.4)
Progressive Disease (PD)	10 (31.3)
Clinical Response	25 (71.4)
No Clinical Response	10 (31.3)

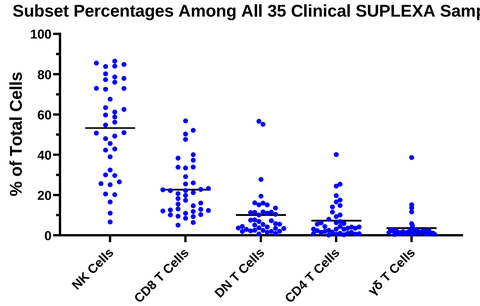
Patient PBMCs analyzed by CyTOF

Patient #	Cancer Type	Clinical Benefit
111	CRC-MSI-H	Yes
117	CRC-MSI-H	Yes
119	CRC-MSI-H	Yes
209	CRC-MSS	No
212	CRC-MSS	No
110	ccRCC	Yes
113	ccRCC	Yes
118	ccRCC	Yes
122	ccRCC	Yes
120	ccRCC	No
112	Melanoma	Yes
215	Melanoma	Yes
301	Melanoma	Yes
115	Lung	Yes
114	Breast	Yes

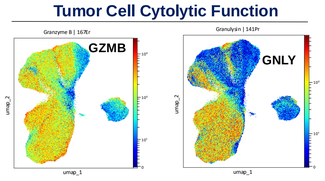
For further information, see accompanying poster, **Poster 608**, Final safety and efficacy update of SUPLEXA-101, a First-in-Human, Single-Agent Study of SUPLEXA Therapeutic Cells in Metastatic Solid Tumors.

SUPLEXA Cell Phenotyping By CyTOF

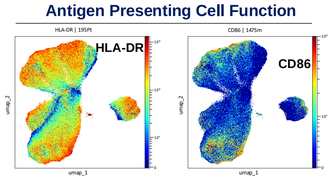
Subset Percentages Among All 35 Clinical SUPLEXA Samples



Tumor Cell Cytolytic Function



Antigen Presenting Cell Function



SUPLEXA Cell Transcriptional Profiles

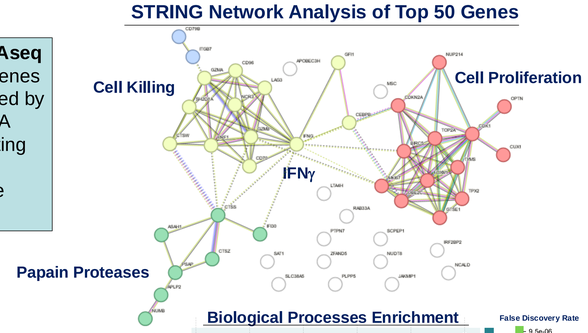
Top 20 Significant Genes	
Gene Symbol	logFC
IFNG	5.62
GZMA	4.94
NUP214	-2.79
APOBEC3H	4.03
NCALD	3.18
PSAP	-3.79
AC092580.4	5.84
CEBPB	-4.00
SAT1	-4.08
OPTN	2.67
UBE2C	7.29
TYMS	6.43
CD79B	-2.81
CTSS	-4.71
TOP2A	6.14
PTPN7	2.71
LTA4H	-3.30
FOXM1	5.81
CDKN2A	4.95
JAKMIP1	3.71

Next Gen Bulk RNAseq Among the top 20 genes up- or down-regulated by >2-Fold in SUPLEXA cells relative to starting PBMC, **IFN-γ** and **granzyme A** are the most significant.

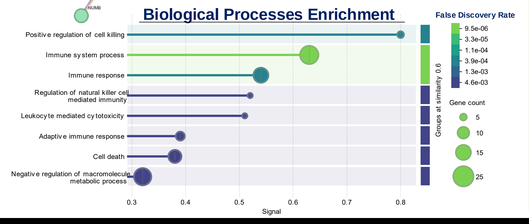
Top 20 Significant Genes	
Gene Symbol	logFC
CCNB2	4.69
TOP2A	4.63
GTSE1	2.93
CDCA8	3.17
TPX2	3.12
ELOVL6	2.55
GZMA	2.23
MKI67	3.15
CCNA2	3.76
CENPA	2.48
KIF20A	2.82
TYMS	3.67
CDC20	3.97
CDCA5	3.20
HJURP	2.58
KIF2C	2.72
GZMB	1.82
NCAPH	2.34
IFNG	2.23
TTK	2.53

Nanostring Technology Among the top 20 genes up-regulated by >2-Fold in SUPLEXA cells relative to starting PBMC, are **granzyme A** and B as well **as IFN-γ**.

STRING Network Analysis of Top 50 Genes



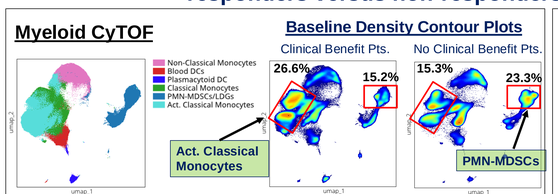
Biological Processes Enrichment



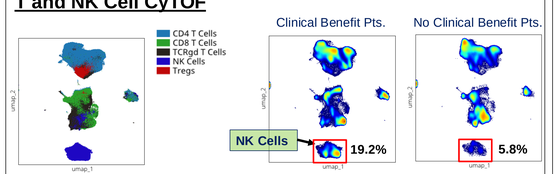
Longitudinal Blood CyTOF Analysis of SUPLEXA-treated Patients

Analysis of baseline PBMCs identifies possible biomarkers discriminating responders versus non-responders by CyTOF

Myeloid CyTOF

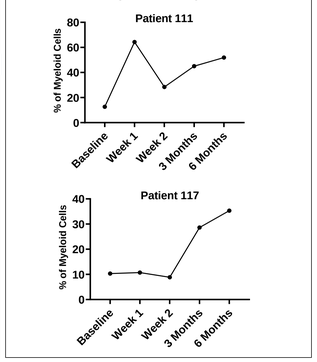


T and NK Cell CyTOF



Longitudinal Myeloid CyTOF: 2 CRC-MSI-H Patients

Activated Classical Monocytes: (SIGLEC-1+)

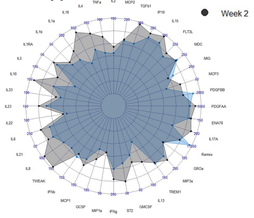


Note: Patient samples demonstrated individualized patterns indicative of the inherent interpatient biologic heterogeneity. These results highlight emerging data to support predictive biomarkers.

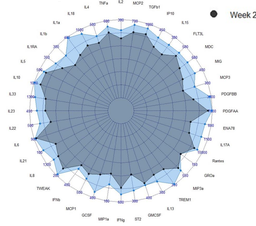
Longitudinal Analysis of Plasma Proteomics by Luminex and Olink

Luminex Plasma Cytokine Profiles

Immune Suppressed Patients Baseline Week 2

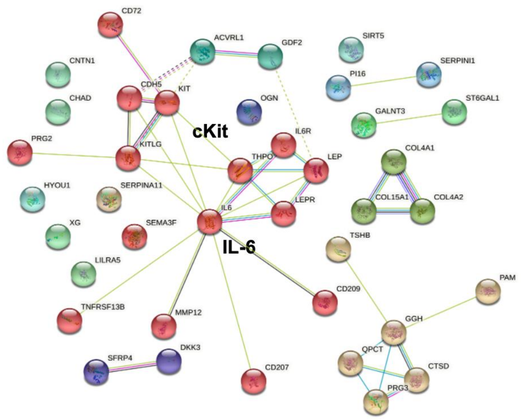


Inflammatory Patients Baseline Week 2



Olink Discovery Plasma Proteomics

STRING network analysis of longitudinal plasma markers significantly changed by SUPLEXA



Summary and Conclusions

- SUPLEXA cells show individualized cell subset profiles with common acquisition of **tumor cytolytic** and **antigen presenting cell** phenotypes
- Transcriptome analysis shows that **cell killing**, **proliferation**, and **papain proteases** are hub gene networks that are altered in SUPLEXA cells
- Baseline immune cell characteristics show striking differences with higher levels of **NK cells** and lower levels of **MDSCs** in patients showing clinical benefit.
- Longitudinal CyTOF analysis PBMCs demonstrates pharmacodynamic increases in **activated classical monocytes (SIGLEC-1+)**.
- Analysis of longitudinal plasma samples reveal modulation of **cytokines** that impact inflammatory cytokine (**IL-6**) and hematopoietic factor (**cKit**) networks.