Alloplex Biotherapeutics

11th Annual
Innate Killer Summit
Advancing NK Cell Therapies & Engagers from Concept to Clinic

March 24-25, 2026 | San Diego, CA
www.innate-killer.com

The versatile ENLIST immune cell training platform,

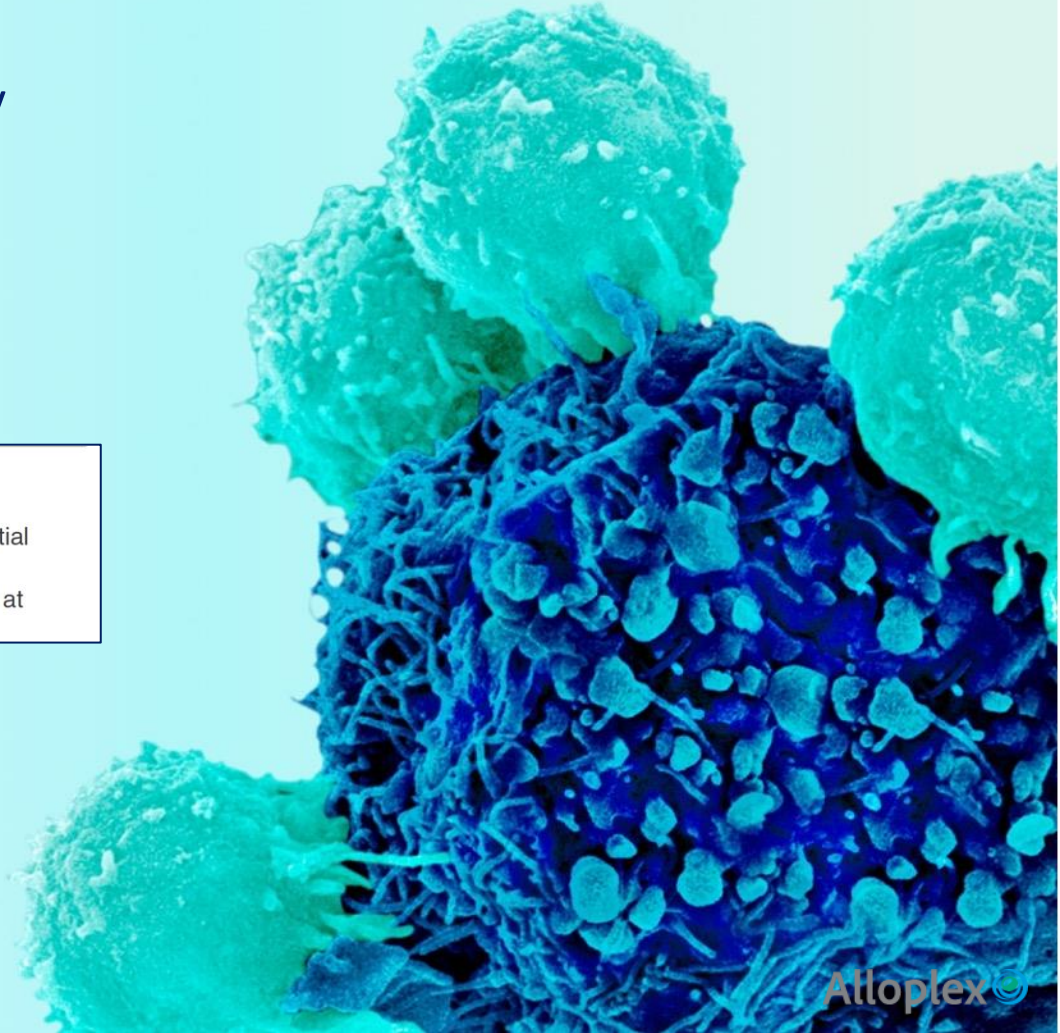
for manufacturing SUPLEXA, is a clinically de-risked cell therapy

with broad application across multiple indications

Frank Borriello
Chief Executive Officer
Alloplex Biotherapeutics

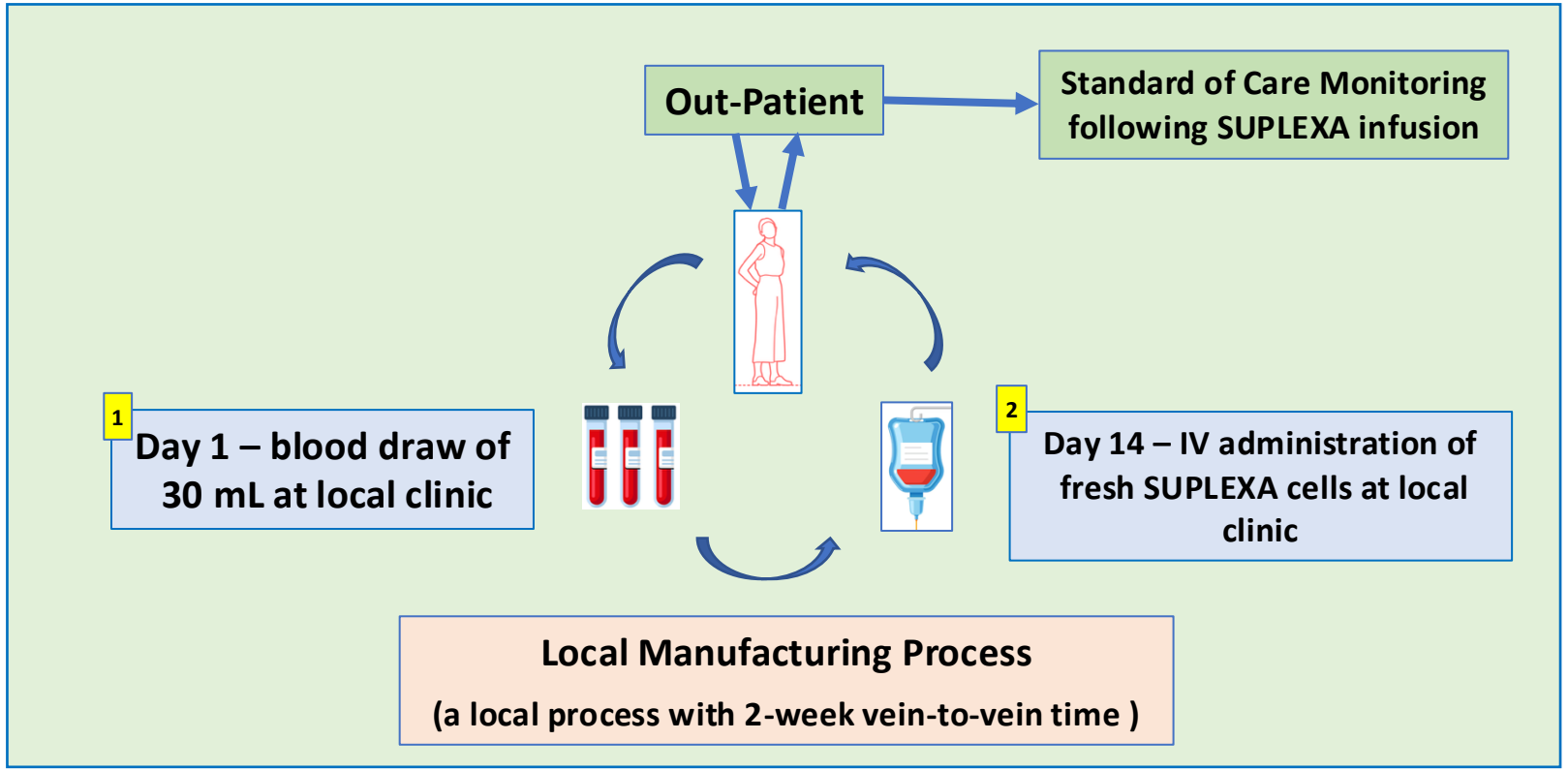
3.30 **Developing Next-Gen NK Cells to Boost Safety & Potency for More Effective Cancer Therapies**

- Developing strategies to enhance NK, persistence, migration, tumor-killing potential and improving safety profiles
- Exploring scalable platforms for making *ex vivo* cell manufacturing more efficient at reduced cost



March 25, 2026

SUPLEXA is a clinical stage cell therapy asset developed with a prioritization of the patient experience



- ***Robust, efficient and cost-effective*** manufacturing.
- ***Pristine safety profile*** permits out-patient treatment.
- ***Low cost of goods*** and favorable pharmaco-economics.
- ***Simplicity*** *is the foundation of a robust, reproducible* and affordable product.

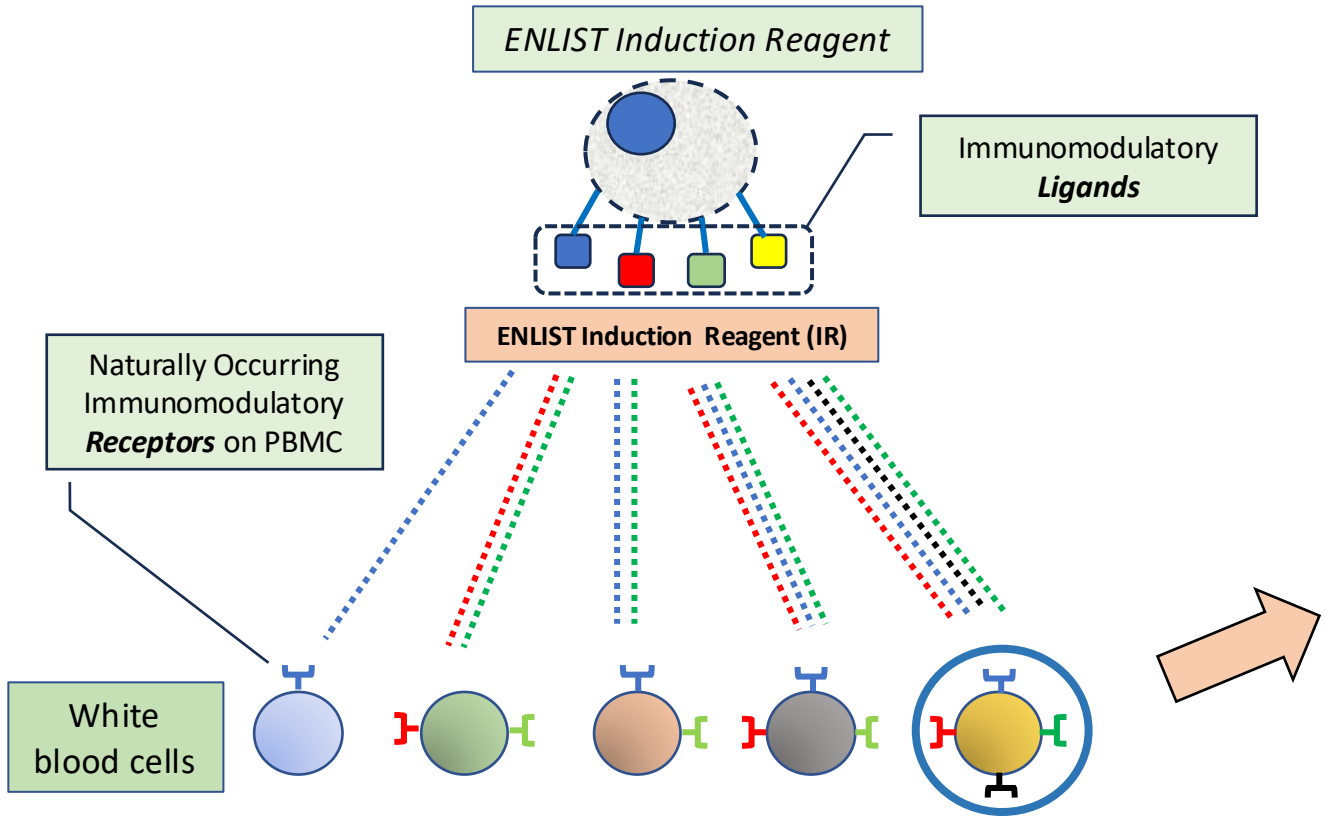
Perennial Considerations

- **Autologous versus Allogeneic**
- **Non-engineered versus engineered**
- **Source of immune cells**
- **Overall efficiency of manufacturing**
- **Safety**
- **Efficacy**
- **Ease of Manufacturing/COGs**
- **Ease of Use**
- **Potential for combination therapy**

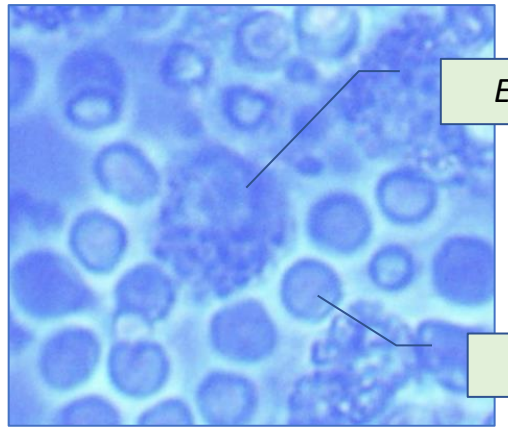
SUPLEXA Features

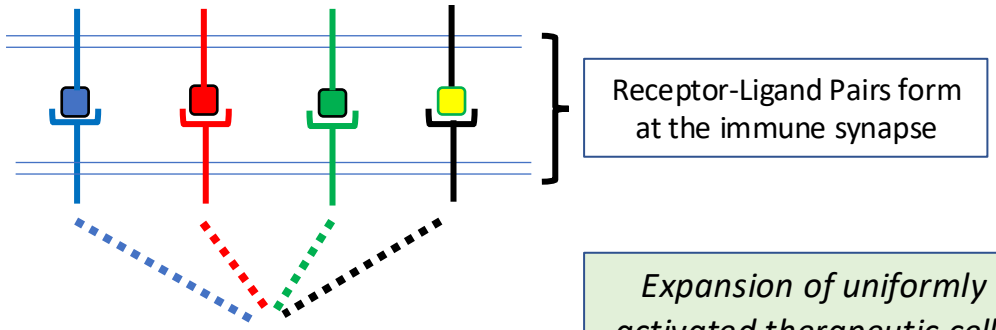
- **Autologous**
- **Non-engineered**
- **Whole blood**
- **100% efficiency observed in Phase 1**
- **Pristine safety observed in Phase 1**
- **Clinical activity observed in Phase 1**
- **Simple robust method with low COGs**
- **Out-patient administration with no chemo preconditioning**
- **Readily used in combination with standard-of-care**

The ENLIST-mediated immune cell training platform underpins the Alloplex pipeline



Note that multiple immune cell sub-types naturally present in PBMC each express a unique array of receptor.

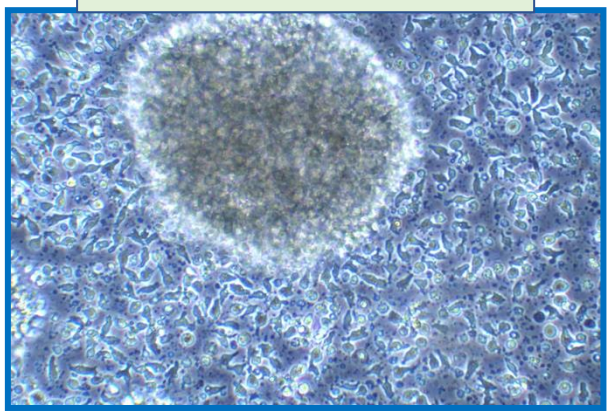




Integration of receptor signaling

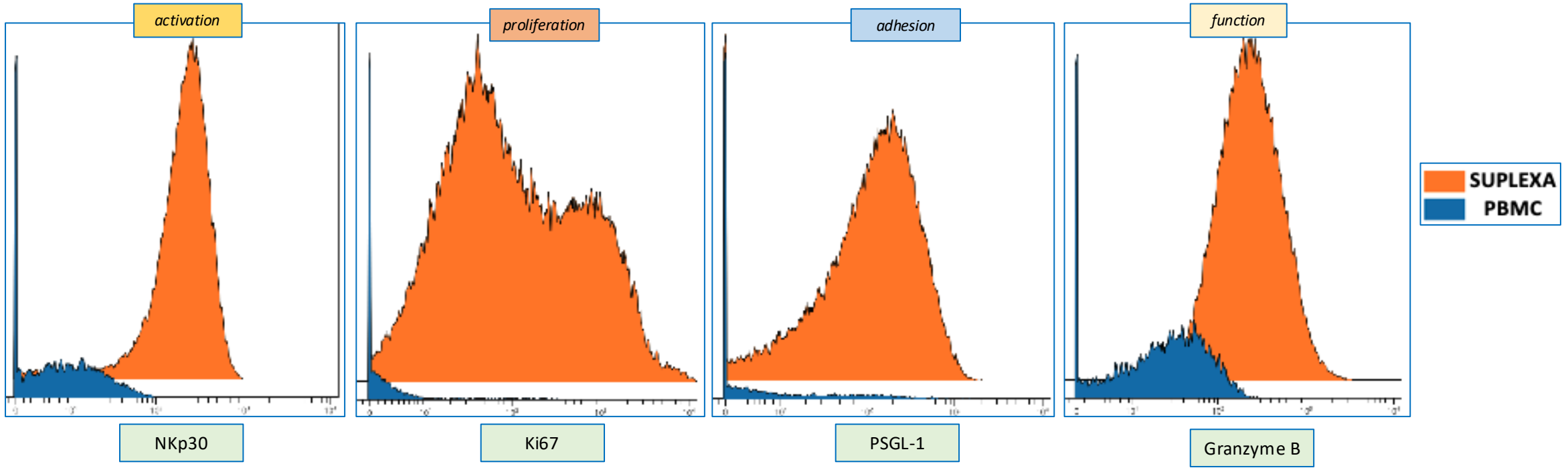
BIOLOGIC RESPONSE
- **Proliferation**
- **Differentiation**
- **Cytokine Production**
- **Tumor cell killing capacity**

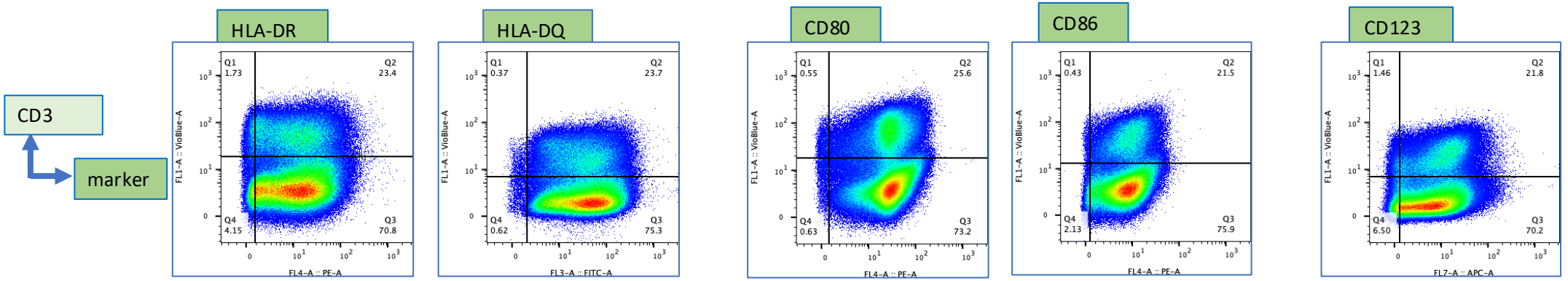
Expansion of uniformly activated therapeutic cells



Alloplex

SUPLEXA characterization reveals presence of cytolytic and APC-like mechanisms

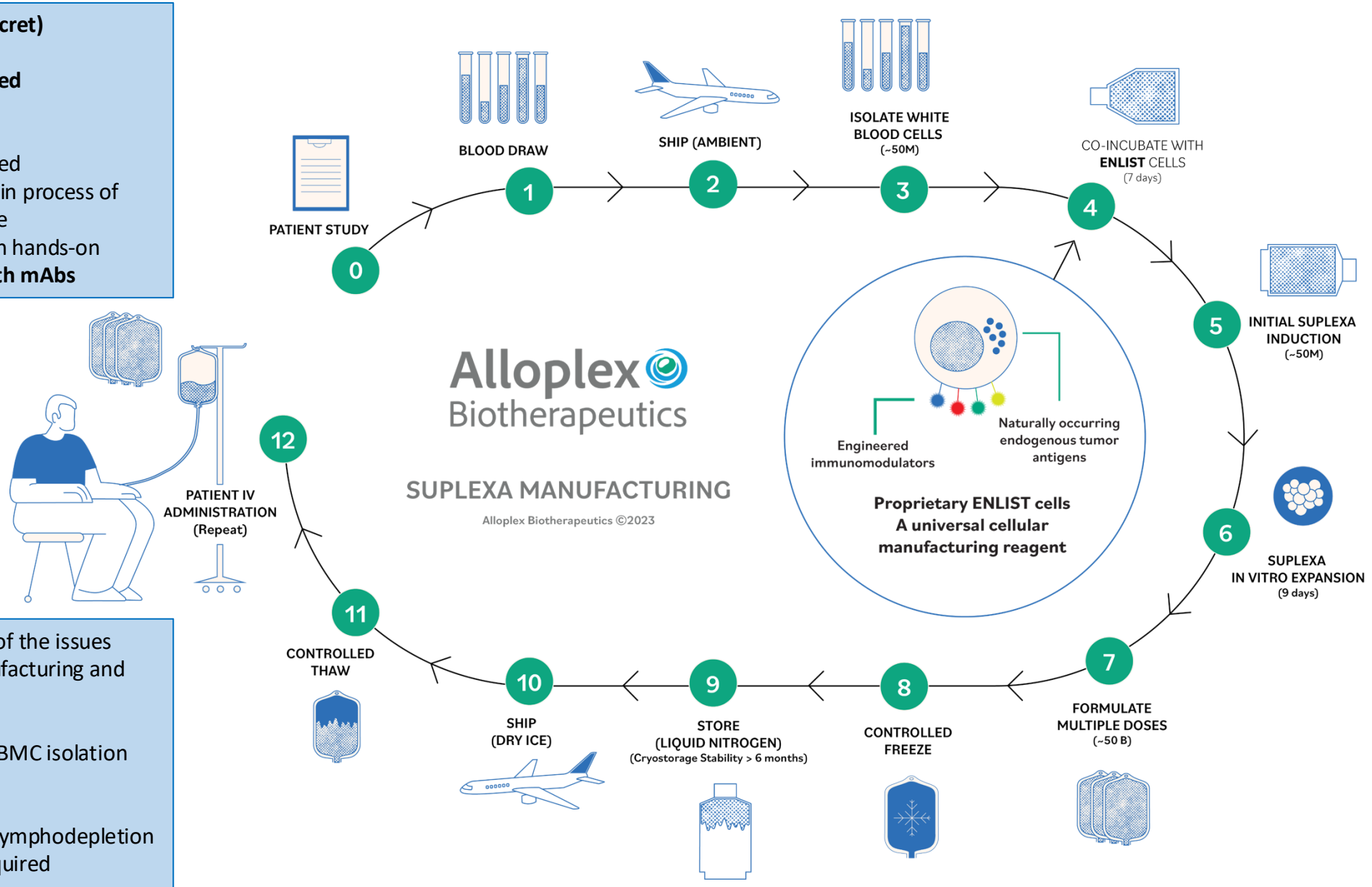




Alloplex

SUPLEXA are made by a clinically validated individualized, robust and reproducible process

- **Proprietary (patent and trade-secret)**
- **GMP grade**
- **Only standard equipment required**
- **Proven transferability**
- **Uniquely commercially scalable**
  - 2 air freight shipments required
  - Currently, a 35-day vein-to-vein process of which 21 days is tissue culture
  - Only 3 days require technician hands-on
- **Cost objection – to be on par with mAbs**



Note: Alloplex has overcome many of the issues associated with Individualized manufacturing and therapeutic implementation.

- No leukopheresis,
  - just venipuncture and PBMC isolation
- No cell selection
- No genetic engineering
- No chemo preconditioning for lymphodepletion
- No clinical cytokine support required
- Multiple doses possible

First reported at
SITC
Houston, TX
Nov 7-10, 2024

Poster: 608

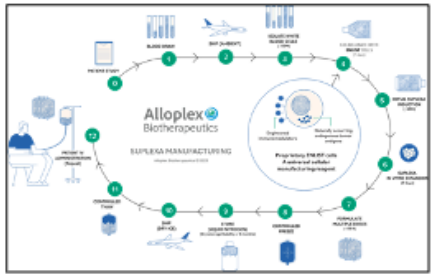
Final safety and efficacy update of SUPLEXA-101, a First-in-Human, Single-Agent Study of SUPLEXA Therapeutic Cells in Metastatic Solid Tumors

[1]R Joshi, [2,3]JC Goh, [2]G Kichenadasse, [2,4]V Atkinson, [1]S Bishnoi, [2]W Joubert, [1]V Kwatra, [1]M Okera, [2]G Nisyrios,[5,6]J Lederer, [6]S Gargosky, [6]F Borriello
[1]Cancer Research South Australia, [2]Gallipoli Medical Research Foundation, [3]Queensland University of Technology, [4]University of Queensland,
[5]Brigham and Women's Hospital, Harvard Medical School, [6]Alloplex Biotherapeutics, Inc., Woburn, MA

Background

SUPLEXA therapeutic cells are an autologous, non-engineered cell therapy derived from patient PBMCs isolated from about 50 mL of whole blood. They are composed of highly activated immune 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'). This first-in human study is a non-comparative, open-label, single-agent survey study designed to address safety and clinical activity in subjects with various solid tumors. Notably, no other active agents were used in these end-stage patients, thus toxicity from chemotherapeutic preconditioning and cytokine support was avoided.

SUPLEXA manufacturing



Note: Only 50 mL of whole blood is required per patient.

Study Design

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 schedule.

Objectives	Endpoints
Assess Safety and tolerability of SUPLEXA in subjects with malignant solid tumors.	•Incidence of DLTs, AEs, SAEs
Assess the efficacy of SUPLEXA based on RECIST evaluation criteria.	•Overall Response Rate (ORR) •Time-to-Progression (TTP)
Clinical Exploratory Endpoints	•Progression - free Survival (PFS) •Duration of Response (DOR) •Time to Response (TTR) •Clinical Benefit Rate (CBR) •Overall Survival (OS)
Scientific Exploratory Studies (results presented in an additional poster)	•Interpolation comparison of SUPLEXA batches. •Evaluation of longitudinal blood samples in individual patients assessing for changes in cellular composition and inflammatory cytokines.

Summary of Demographics and Baseline Characteristics

All patients had exhausted standard therapeutic options including chemotherapy and immune checkpoint inhibitors (ICIs).

Parameter	Statistics	Solid tumor Pts
Age	(N), Mean (SD)	(35), 63.6 yrs (10.1)
Gender	Male, Female	18, 17
Race	Caucasian, Asian	31, 4
ECOG Status	(Total), 0, 1, 2, 3, 4	(35), 24, 11, 0, 0, 0

Safety (treatment emergent adverse events)

Parameter	Statistics	Solid Tumors
Total subjects	N	35
Subjects with at least one TEAE	N, Not-related (NR), Related (R)	25, 22, 3
Subjects reporting TEAEs by severity	Total, grade 1, 2, 3, 4, 5	25; 9, 10, 3, 2, 1
Subjects with at least one serious TEAE	Affected subjects, Gastrointestinal disorders, Infections, Musculoskeletal, Respiratory	6 4 1 1 2
Subjects reporting drug related TEAEs	Subjects with at least 1 related TEAE; grade 1, 2, 3, 4, 5 Musculoskeletal, (N; grade 1, 2, 3, 4, 5) Arthralgia, (N; grade 1, 2, 3, 4, 5) Back Pain, (N; grade 1, 2, 3, 4, 5)	3; 1, 2, 0, 0, 0 2; 1, 1, 0, 0, 0 1; 1, 0, 0, 0, 0 1; 1, 0, 0, 0, 0

Note: Critically, there were no DLTs identified, no injection site reactions, no serious drug related adverse events. no laboratory abnormalities, no abnormalities in vital signs and ECGs

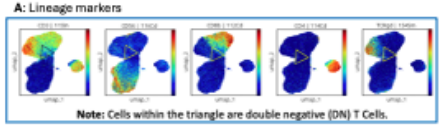
Best Overall Response and Event-free Survival

Best Overall Tumor Response	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)

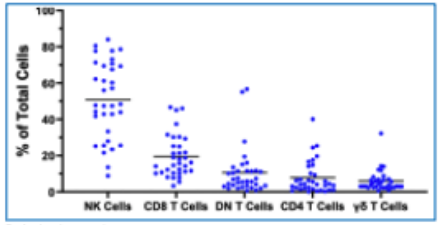
	Statistics	Solid Tumors
Descriptive	N Event Censored	32 19 13
Time to Event (weeks)	Median (95% CI)	19.3 (7.1, NE)
Event-free Rate (%) (95% CI)	12 weeks 24 weeks 36 weeks 48 weeks	61.2 (41.8 -75.1) 36.9 (19.3 -54.6) 32.8 (16.1 -50.6) 32.8 (16.1 -50.6)

Composite CyTOF Data for 35 SUPLEXA Clinical Batches

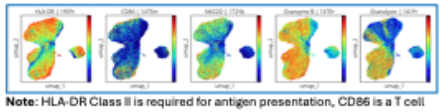
A: Lineage markers



Note: Cells within the triangle are double negative (DN) T Cells.

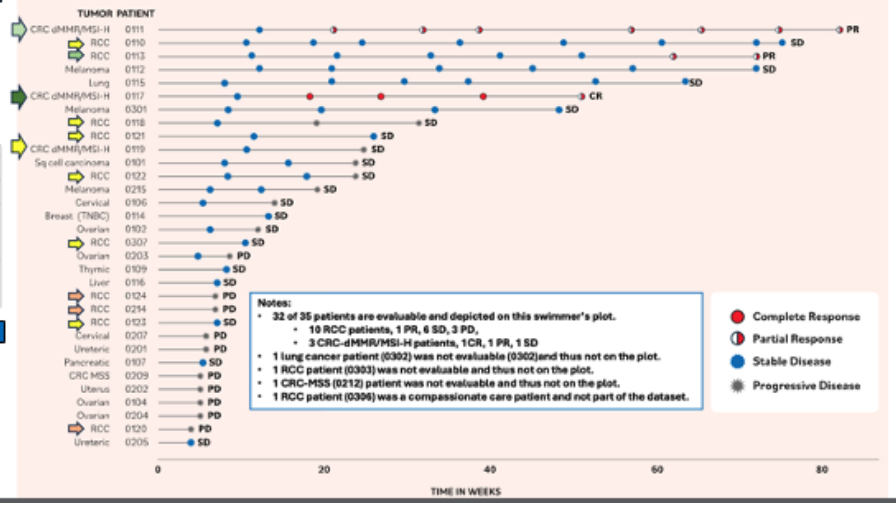


B: Activation markers

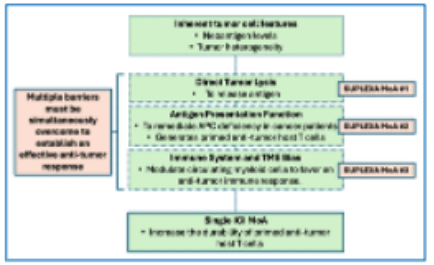


Note: HLA-DR Class II is required for antigen presentation, CD86 is a T cell costimulatory molecule, NKG2D is an NK activation molecule involved in cytolysis, Granzyme B and Granulysin are cytolytic proteins.

Swimmers Plot and Best Overall Clinical Responses



SUPLEXA mechanisms provide a strong rationale for synergy with immune checkpoint inhibitors



It is widely accepted that the key mechanism by which ICI act on the immune system is by blocking the activation of a checkpoint protein (e.g. PD-1 and CTLA4) which would otherwise down-regulate the anti-tumor host T cell response. This means that ICI are dependent on the availability of anti-tumor host T cells for their activity. As cancer patients have a known defect in the antigen presenting cells that prime anti-tumor host T cells, this may provide a bottle neck which when combined with an abundance of suppressive Tregs and MDSC, restrains a functional anti-tumor immune response. We hypothesize that the multiple mechanisms of SUPLEXA shown above, are complementary to that of ICIs and should result in an increased availability of primed anti-tumor host T cells and an alteration in the myeloid compartment that results in an immune environment more permissive to their function.

Conclusions

All study endpoints were achieved. Safety was established over a wide dose range (3-20 doses of 2.5B cells per patient) and a total of >220 administered doses. No related DLTs, injection site reactions or drug related serious adverse events were identified throughout the course of the study. Signs of clinical efficacy were demonstrated with a CR and two PRs and a number of long-lasting SD responses in various tumor types including, CRC-dMMR/MSI-H, ccRCC, melanoma, lung cancer and TNBC.

Based on these positive first-in-human single-agent clinical trial results in select tumor types – supported by laboratory evidence that SUPLEXA cells modulate the immune environment of treated patients – a Phase 2 study of SUPLEXA combined with ICIs in front-line CRC-dMMR/MSI-H patients is under development. Since SUPLEXA possesses APC-like properties, we hypothesize that SUPLEXA cells may facilitate the production and function of anti-tumor primed T cells. Since the mechanism of action for immune checkpoint inhibitors depends on the presence of such primed T cells, we suggest that a combination of SUPLEXA with ICIs may result in synergistic activity with an improvement in the current 12-month PFS of ~55%.

This Phase 2 study will be open label 2-arm comparing the standard of care ICI against ICI combined with SUPLEXA. The advantage of such a study is that all participants receive ICI standard of care, and as front-line patients are less fragile.

For further information, see accompanying poster, Poster 378, 'Transcriptional and proteomic insights into the immunomodulatory nature of SUPLEXA cells: An autologous cellular therapy for cancers'

Acknowledgments

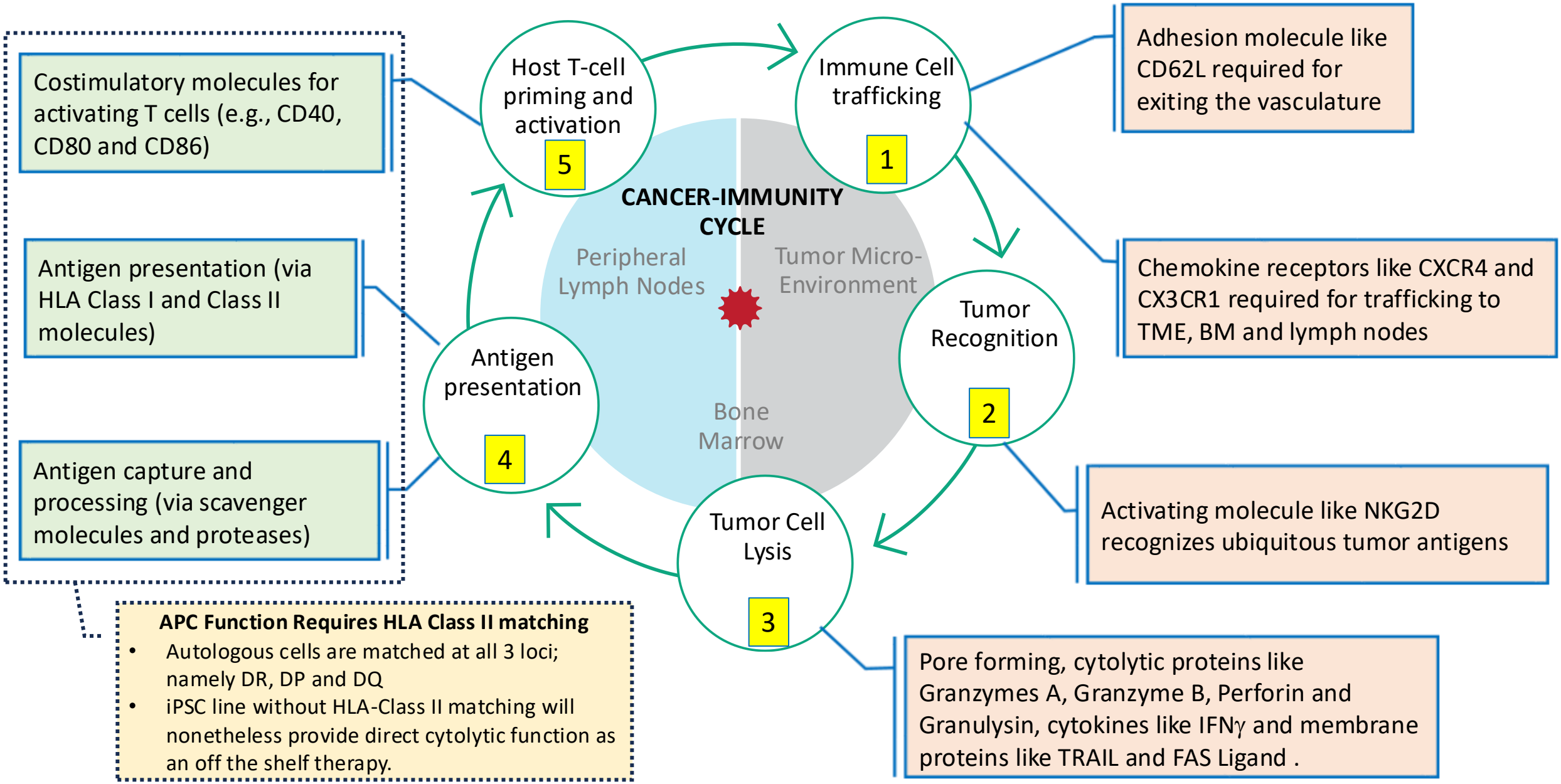
We thank the patients, their families and our clinical partners, for their support Syner-G BioPharma Group, QIMR Berghofer Medical Research Institute (QGEN Cell Therapeutics in Brisbane AUS), Novotech (Sydney, AUS) for Trial Oversight.

Note 1: poster available for download from Alloplex website.

SUPLEXA Therapeutic Cells: the first cell therapy candidate to emerge from the ENLIST immune cell training platform

- **Lead program** - autologous (self), non-engineered, PBMC-derived option.
  - Unbiased approach requiring no cell selection.
  - Avoids the introduction of immunogenicity through expression of a foreign protein which could be recognized by the host and negatively impact cell persistence and durability.
  - Follows an established regulatory paradigm.
- **Manufacturing** - Requires only ~50 mL of whole blood and 35-day vein-to-vein time.
  - Highly scalable manufacturing with multiple infusions made from a single manufacturing batch.
- **Uniquely activated immune cell product** comprised predominantly of NK- and T- lineage cells.
  - High levels of HLA Class II, T cell costimulators, cytolytic proteins, activation proteins, cytokines and chemokines with their associated receptors.
  - Notably devoid of exhaustion markers such as PD-1, CTLA4 and NKG2A checkpoints
- ***Single–agent Phase 1 study in metastatic solid tumor patients achieved all prespecified endpoints***
  - **Entry Criteria** - Patients had exhausted all standard options including chemo and checkpoint inhibitors.
    - No chemo preconditioning or supportive cytokines were used.
  - **Safety** – pristine profile (only several transient Grade 1/2 adverse events).
  - **Efficacy** – activity in multiple solid tumor types with durability.
  - **Exploratory** - pharmacodynamic biomarkers identified in myeloid blood composition.
- **Multiple potential mechanisms** of activity have been identified for SUPLEXA cells.

SUPLEXA cells act by multiple mechanisms at discrete steps in the cancer-immunity cycle

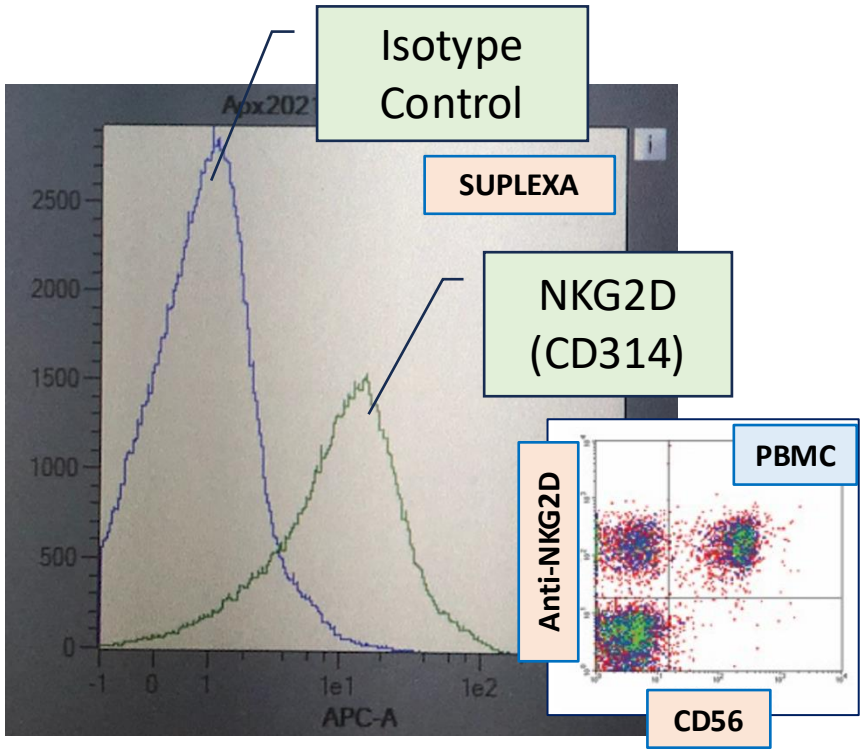


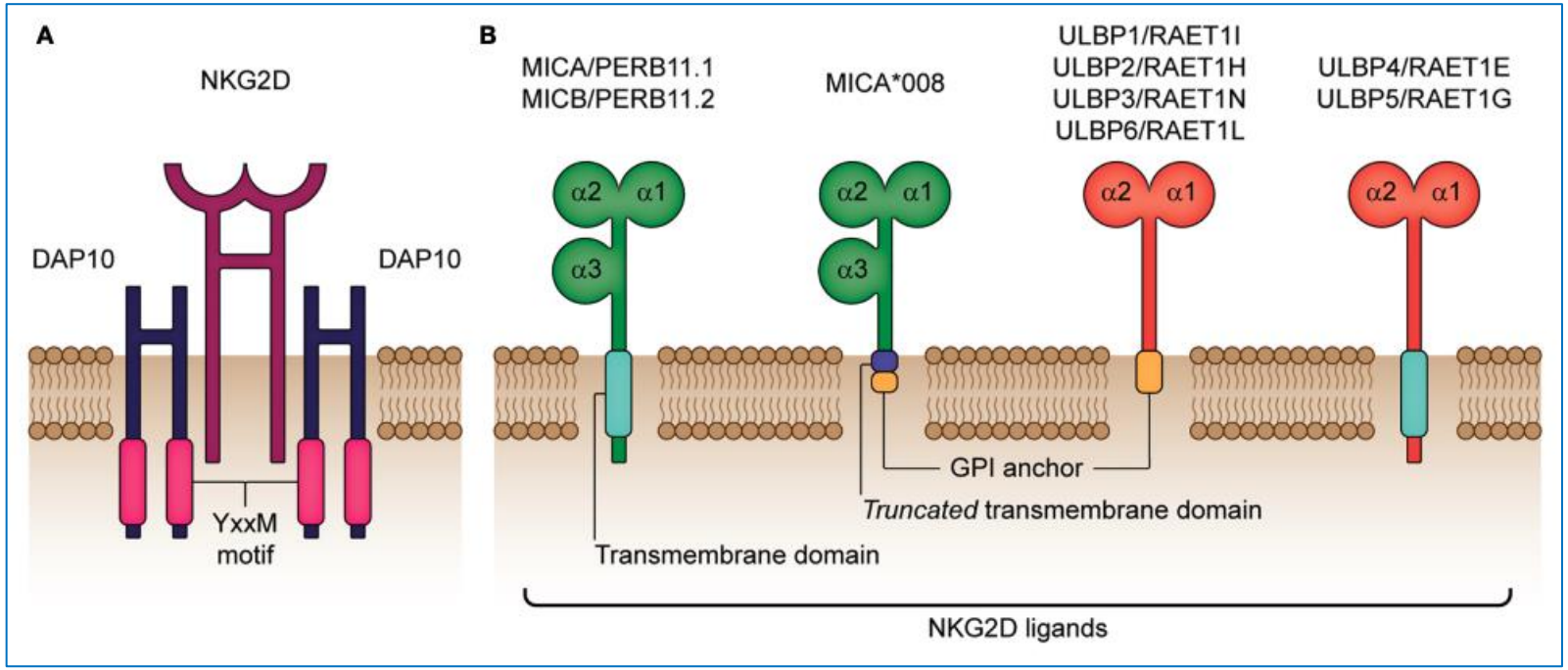
APC Function Requires HLA Class II matching

- Autologous cells are matched at all 3 loci; namely DR, DP and DQ
- iPSC line without HLA-Class II matching will nonetheless provide direct cytolytic function as an off the shelf therapy.

SUPLEXA cells target tumor cells, in part, via expression of the germline encoded NKG2D receptor

NKG2D is an activation receptor that detects stress ligands that are ubiquitously expressed on tumor cells



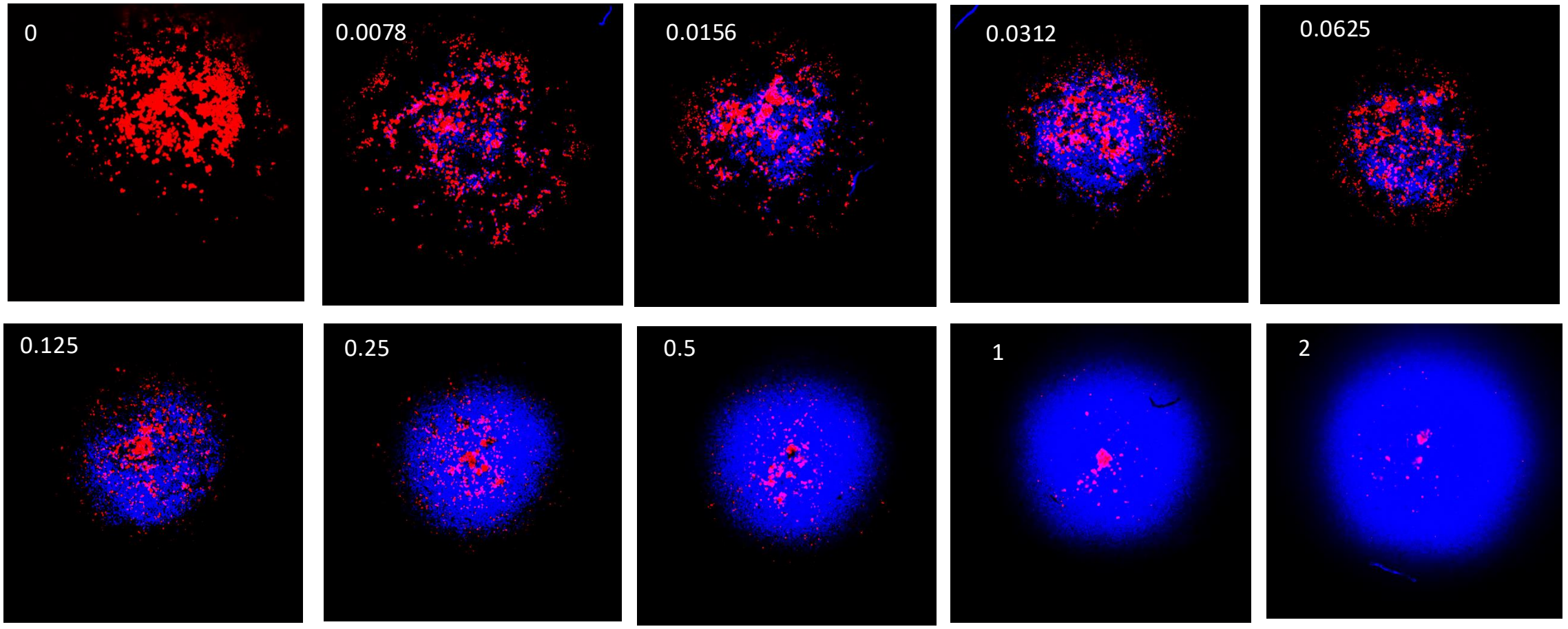


NKG2D (stress) ligands are inducible by

- Tumor transformation (cancer cells)
- Acute Damage (infection)
- Chronic damage by age (senescent cells)

NSCLC PDX Organoid Data

CTG-3651 SUPLEXA Cells 24h treatment; similar results observed for a CRC PDX model



Live PDX Tumor Cells

Live SUPLEXA Cells

- **Representative images from Day 4 of the assay (Images after 24 hours of co-culture)**
- Representative images from each of the 9-cell concentration tested are shown here with cell number (in millions) in the top-left corner at 4x magnification. Contrast has been adjusted to optimally depict representative images at higher cell doses, but uniform settings were used throughout for analysis.

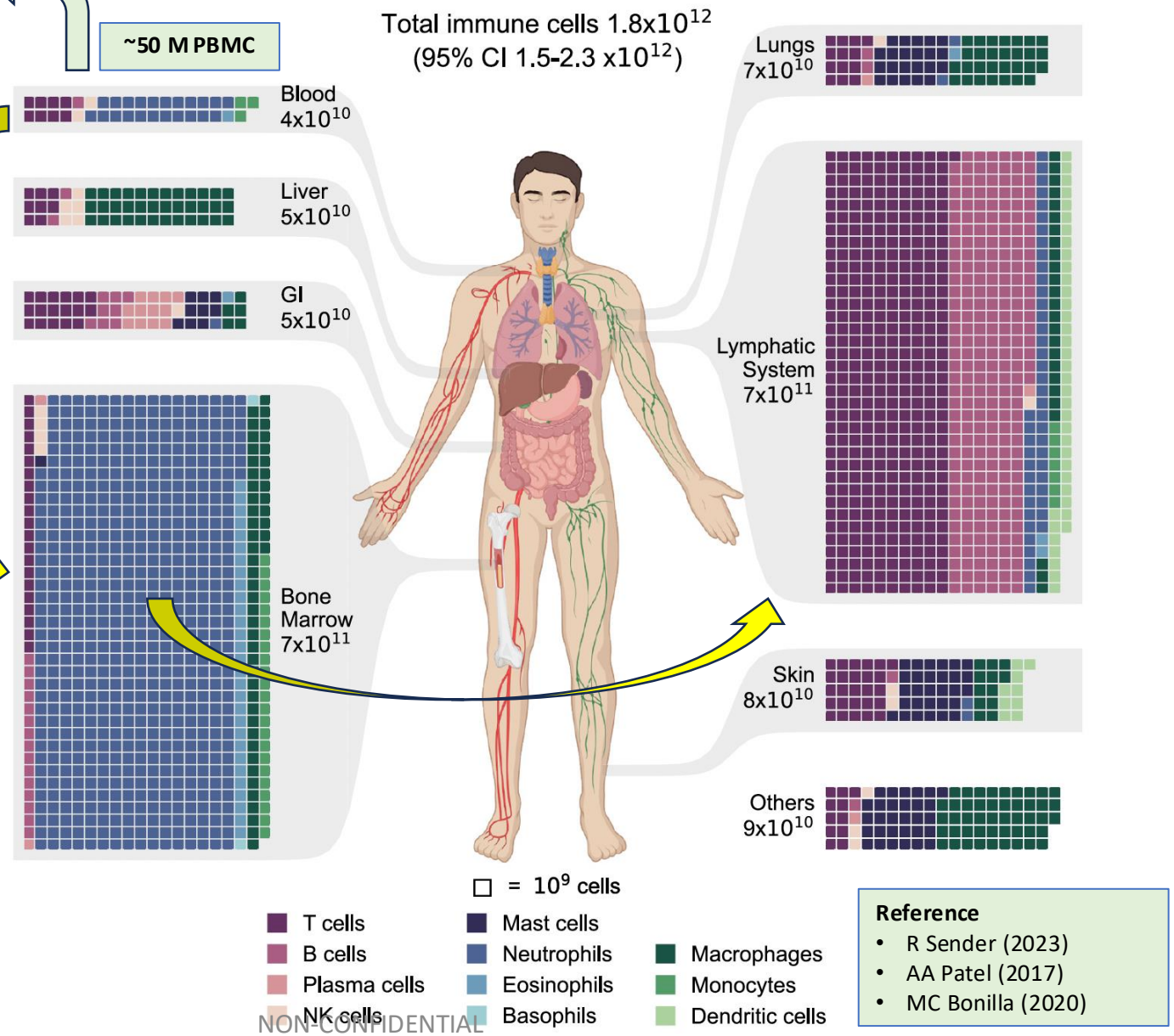
Perspective on SUPLEXA cell number relative to host immune cell populations

SUPLEXA manufacturing

~50 M PBMC

Target SUPLEXA dose (4 x 10 B cells)

(~6-10% of bone marrow cell number)



CXCR4 and CXCR3 chemokine receptor expression on SUPLEXA cells allow them to traffic to the bone marrow and lymph nodes

Bone marrow in MM patients is also the tumor microenvironment (TME) where the tumor stem cells reside, hence the effector and target cells are colocalized for maximal anti-tumor effect through contact dependent mechanisms.

Reference

- R Sender (2023)
- AA Patel (2017)
- MC Bonilla (2020)

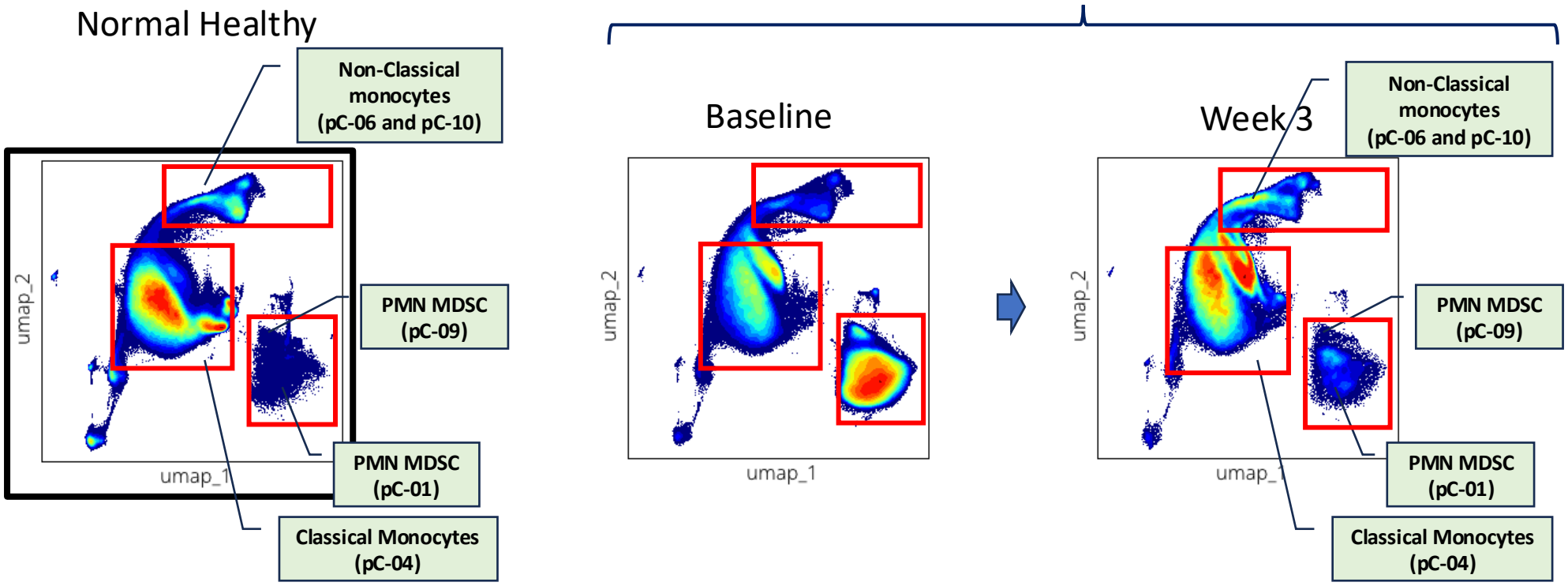
Alloplex

SUPLEXA induces a pharmacodynamic alteration in the peripheral blood of treated patients suggesting bone marrow modulation

- Myeloid derived suppressor cells (MDSCs) are short lived cells capable of potently suppressing host immune responses.
- An accumulation of MDSC in the bone marrow of MDS patients is observed and believed to be responsible for degradation of bone marrow function and linked to poor prognosis.
- Elevated MDSC levels are also considered a major contributor to immune checkpoint inhibitor (ICI) failure.
- SUPLEXA cells have the potential to reduce MDSC levels in the bone marrow and peripheral blood of MDS patients.

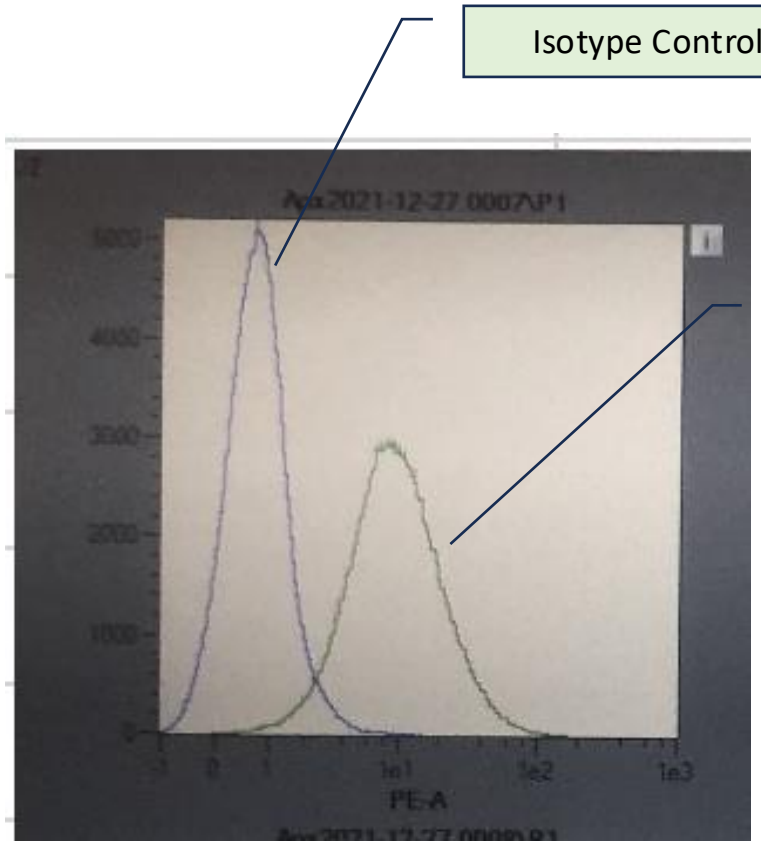
Contour Plots Showing Treatment Response

(patients with high initial MDSC counts in PBMC, n=10)



Alloplex

CXCR4 Expressed on SUPLEXA Cells Enables Migration to the Bone Marrow



- CXCR4 is uniformly expressed on ~100% of SUPLEXA cells.
- CXCR4 activation potentiates chemotactic activity in various cell types
- CXCL12 (SDF-1) is the only known ligand for CXCR4
- CXCL12 (SDF-1) is produced by stromal cells in the bone marrow as well as fibroblasts that comprise the bulk of the tumor microenvironment (TME).

Rationale for the Prioritization of Hematologic Malignancies

- ***in vitro***, SUPLEXA cells are cytolytic against a broad array of tumor cells upon contact.
- ***in vivo***, SUPLEXA cells can traffic to the bone marrow and lymph nodes putting them in juxtaposition to tumor cells residing in those locations – this is due to their expression of well-understood chemokine receptors.

Key question – Can SUPLEXA cells be made from the blood of patients with hematologic malignancies?

AML patient PBMC samples already proven to work using second-gen T-PLEXA-O process (poster below)

Alloplex Biotherapeutics

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

[1]F Borriello, [1,2]J Lederer
[1]Alloplex Biotherapeutics, Inc., Woburn, MA, [2]Brigham and Women's Hospital, Harvard Medical School

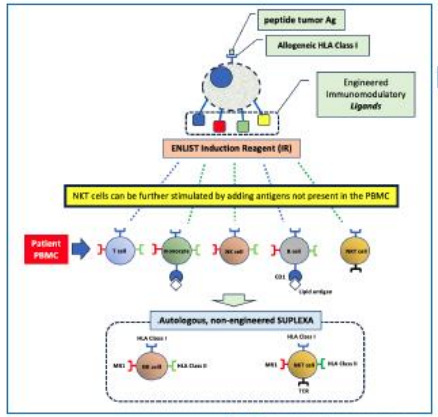
HARVARD MEDICAL SCHOOL

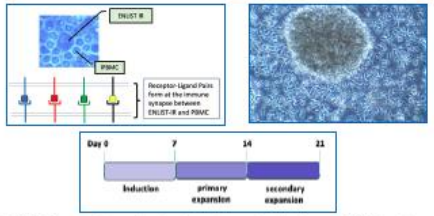
Background

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.

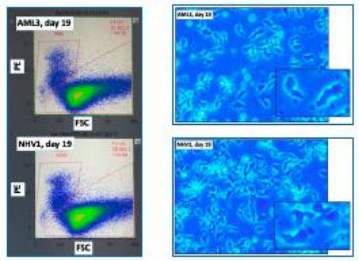
SUPLEXA manufacturing





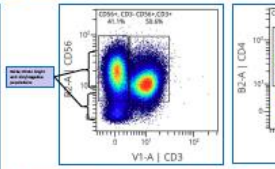
SUPLEXA manufacturing involves the coincubation of PBMC with ENLIST; a tumor cell line modified to express a proprietary combination of membrane expressed immunomodulatory factors. SUPLEXA cells are induced and expand within a 21-day culture period. The system has been optimized for maximal proliferation,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

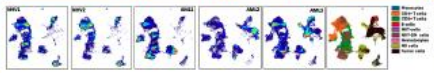
SUPLEXA is comprised of NK and NKT cells

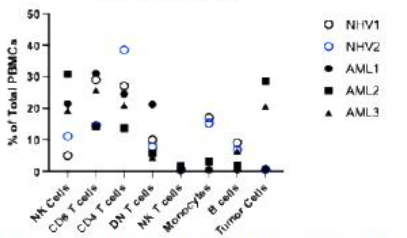


	CD3-	CD3+	CD4+	CD8+	CD4-/CD8-
CD56-	~8%	0%	+++++	+++++	++
CD56+	41%	51%	34%	53%	16%

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.

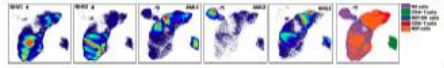
CyTOF of starting PBMC samples

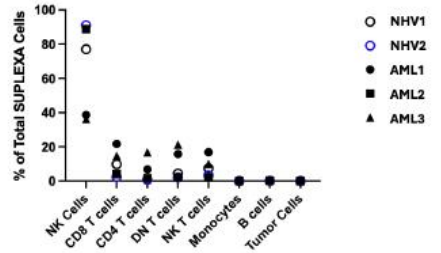




CyTOF analysis of starting PBMC samples from NHV and AML patients with tabulation of major lineages. AML starting PBMC samples contain more NK cells, fewer monocytes and variable numbers of tumor cells.

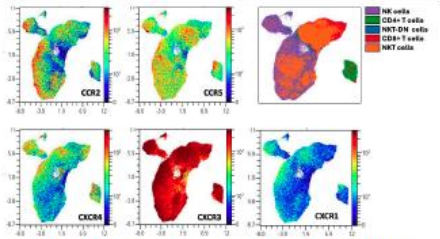
CyTOF of resulting SUPLEXA cells





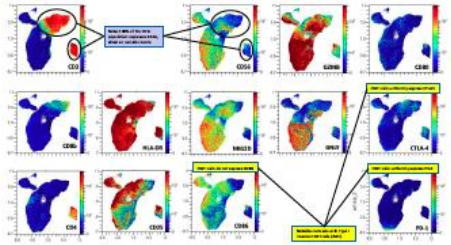
CyTOF analysis of the resulting SUPLEXA cells and tabulation of major lineages. All SUPLEXA cell preparations show similar immune cell types.

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

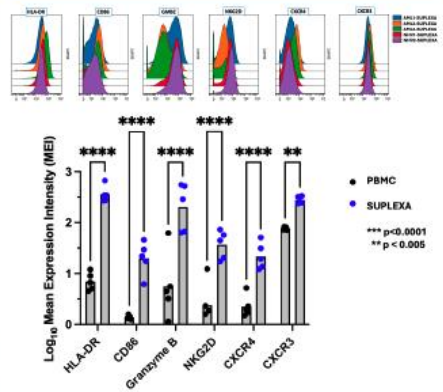


CyTOF analysis of the resulting SUPLEXA cell marker distribution. SUPLEXA NKT cells are CD3, HLA-DR, CD25 and CD86 positive, as well as both CD4 and CD8 single positive or double negative. SUPLEXA NK cells are all CD56, CD25, CD86, NKG2D, HLA-DR, and Granzyme B. NK cells are also positive for Perforin (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.**

Note: This study is being extended with a collaboration with the DFCI.

Alloplex

CLL patient PBMC samples already proven to work using first gen SUPLEXA process (poster below)

HARVARD MEDICAL SCHOOL

Dana-Farber Cancer Institute

Engineered immunostimulatory cells can convert PBMCs from chronic lymphocytic leukemia (CLL) patients into potent tumor killing immune cells

Joshua W. Keegan, BS[1], Frank Borriello, MD, PhD[2*], Stacey M. Fernandes, BS[3], Jennifer R. Brown, MD, PhD[3] and James A. Lederer, PhD[1*]
[1]Brigham and Women's Hospital and Harvard Medical School, [2]Alloplex Biotherapeutics, Inc., Woburn, MA, [3]Dana Farber Cancer Institute and Harvard Medical School.
*jlederer@bwh.harvard.edu or fborriello@alloplexbio.com

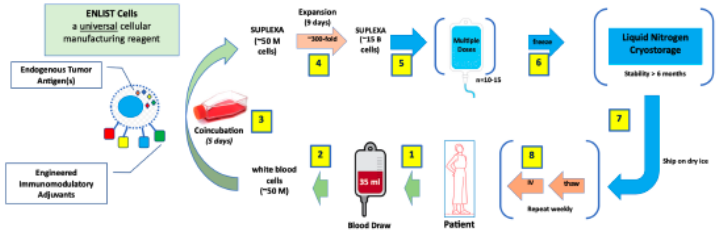
Alloplex Biotherapeutics
engineering advanced cellular therapies

ABSTRACT #7517

Background

A unique autologous cellular therapeutic (**SUPLEXA**) has been developed from human PBMC. It is comprised of NK cells, NKT-like 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 xeno-free manufacturing procedure employing two proprietary engineered leukocyte stimulator cell lines (**ENLIST**) that express an array of immunomodulatory proteins. This process leads to a 300-fold expansion of NK cells, CD8+ T cells, NKT-like cells, and TCRγδ T cells that are called SUPLEXA cells, which will be cryopreserved, and then transferred back into patients as an autologous immune cell therapy for cancer. In this study, PBMCs from CLL patients were used to generate SUPLEXA cells as a first approach to comparatively profile SUPLEXA cells from cancer patients and normal healthy volunteers (NHVs).

The SUPLEXA cell manufacturing process uses 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 novel adoptive cellular immunotherapy for cancer is projected to begin later this year.



Methods

ENLIST cells: Engineered SK-MEL2 melanoma cell lines that express curated sets of > 20 different immunomodulatory proteins that are engineered for membrane expression.

SUPLEXA cell generation: Two million (M) PBMCs isolated from direct blood draws from 10 CLL patients or 5 NHVs were incubated with 0.4 M freeze/thaw killed ENLIST cells for 5 days in XVIVO-15 medium with 2% heat-inactivated human AB serum (XAB2) and then split 1:15 in XAB2 containing IL-7 and IL-15 to expand. 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 an analysis workflow of dimensional reduction by PCA embedded opt-SNE using OMIQ

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. M14 melanoma cells that express red fluorescent protein (RFP) were used as tumor cell targets for these studies. Cytolysis was measured at 48 hours.

Cytokines: A 33 cytokine Luminex panel was used to assess cytokine levels in tumor cell cytolysis supernatants.

CyTOF Antibody Panel

Marker	Metal	Role
CD45	089Y	Pan-leukocyte marker
CD172ab	151Eu	Myeloid cell marker
CD8a	152Gd	CD8 T cell alpha-chain
CD20	153Eu	B cell marker
CD4	154Gd	CD4 T cell marker
CD3	156Gd	All T cell marker
DNAM1	158Gd	Adhesion molecule for NK cell activation
Granzyme*	141Pr	Effector cell tumor killing
KIR2DS1	142Nd	NK receptor that interacts with DAP12
PD-1	143Nd	Activation and checkpoint inhibition
DAP12	144Nd	Signaling adaptor of NK cell activation
CD19	145Nd	B cell marker
CD14	146Nd	monocyte and macrophage
CD84	147Sm	costimulation
Granzyme A	148Nd	Effector cell tumor killing
Granzyme K	149Sm	Effector cell tumor killing
CD28	150Nd	Dipeptidyl peptidase
TCR Vd1	151Eu	gamma delta T cell receptor
CTLA-4	152Sm	Checkpoint inhibition
CD69	153Eu	Lymphocyte activation marker
TCRgd	154Sm	gamma delta T cell identification
ICOS/CD278	155Gd	TF for effector lymphocyte function
CD45	156Gd	CD45 T cell beta-chain
CD16	157Gd	NK marker
CD39	158Gd	ATP to Adenosine enzyme
PLZF	159Tb	Transcription factor, regulates NK cells
NKp30	160Gd	NK cell marker
P-SGL1	161Dy	Adhesion molecule for tissue infiltration
CD56	162Dy	Pan-NK cell marker
4-1BB	163Dy	T cell costimulation
GITR	164Dy	Glucocorticoid-induced receptor - Tregs
NKG2A	165Ho	Effector NK cell marker - negative
CD107a	166Er	Degranulation marker
Gal 9	167Er	Effector cell tumor killing
NKp46	168Er	Activating receptor on NK cells
TCRVa7.2	169Tm	Invariant NKT cells
PDL1	170Er	PD-1 ligand for checkpoint inhibition
TCR-Vd2	171Yb	gamma delta T cell receptor
NKG2D	172Yb	Effector NK cell marker - positive
TCRab	173Yb	alpha-beta T cell receptor
KIR7	174Yb	Recently proliferated cell marker
Tbet	175Lu	TF for NK and effector lymphocyte subsets
Perforin	176Yb	Effector cell tumor killing
CD57	186Pt	T cell exhaustion marker
HLA-DR	195Pt	Histocompatibility
SLAMF6	196Pt	Trigger cytolytic activity of NK and NKT cells
CD58	198Pt	NK adhesion to endothelium
TIGIT	209Bi	Inhibitory receptor on Tregs

* Highlighted markers are shared for intracellular detection

Results

Figure 1: SUPLEXA Cell Manufacturing and Study Design. ENLIST 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 to illustrate the strong immune reaction induced by ENLIST cells

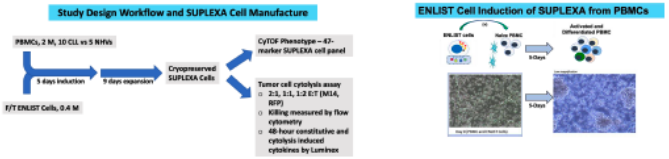
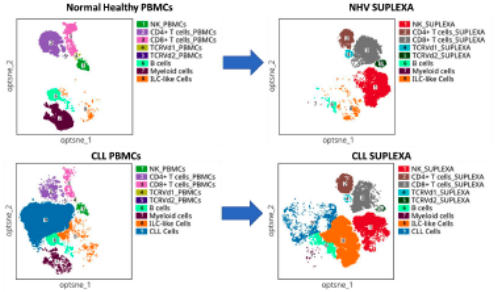
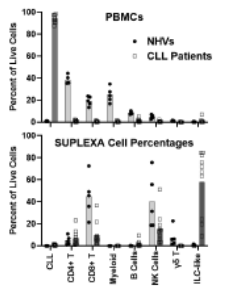


Figure 2: SUPLEXA Cell Phenotyping By CyTOF: SUPLEXA cells from CLL patients and NHVs were analyzed to compare single-cell phenotypes using a customized 47-marker CyTOF antibody panel to identify NK cell and T cell subsets as well as transcription factors, adhesion markers, and functional molecules. Our CyTOF data analysis workflow, cell subset overlay plots of SUPLEXA differentiation of PBMCs from NHVs vs. CLL patients, and cell marker expression profiles of SUPLEXA from NHVs and CLL patients for comparison are shown. Results indicate that SUPLEXA cells can be generated from CLL PBMCs that contain 90% CLL cells.

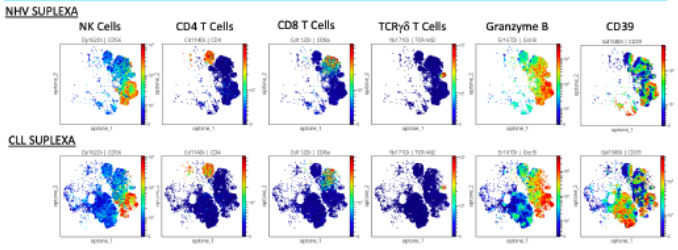
CyTOF Phenotyping of NHV and CLL PBMC SUPLEXA Differentiation



Cell Subset Percentages



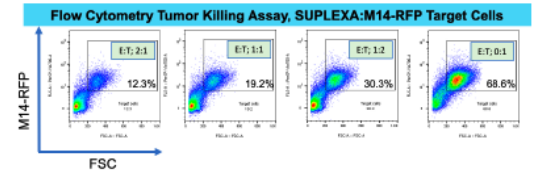
Cell Marker Expression Profiles on SUPLEXA from NHVs and CLL Patients



Results

Figure 3: Tumor Cell Cytolysis Assay and Killing Activity. SUPLEXA cells from NHVs and CLL patients were compared for tumor cell cytolytic activity against fluorescent tumor target cells using a flow cytometry method. Figures show representative killing of M14-RFP target cells and comparative tumor cytolysis activity SUPLEXA from 2 NHVs and 10 CLL patients. Results show potent and similar tumor cytolytic activity between NHV and CLL SUPLEXA cells.

Flow Cytometry Tumor Killing Assay, SUPLEXA:M14-RFP Target Cells



Comparison of NHV and CLL SUPLEXA Cytolytic Activity

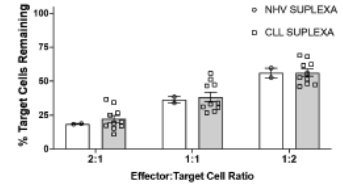
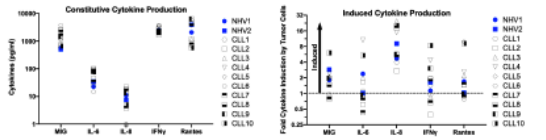


Figure 4: Cytokine Production by SUPLEXA During Tumor Cytolysis. SUPLEXA cells were incubated without or with M14 tumor cells for 48 hours. Supernatants were tested for cytokines by 33-plex Luminex. Plots of constitutive and tumor cell (M14) induced cytokine production by SUPLEXA from NHVs and CLL patient are shown. Results show comparable levels of cytokine production between CLL and NHV SUPLEXA cells.

Constitutive and Induced Cytokine Production by NHV and CLL SUPLEXA



Conclusions

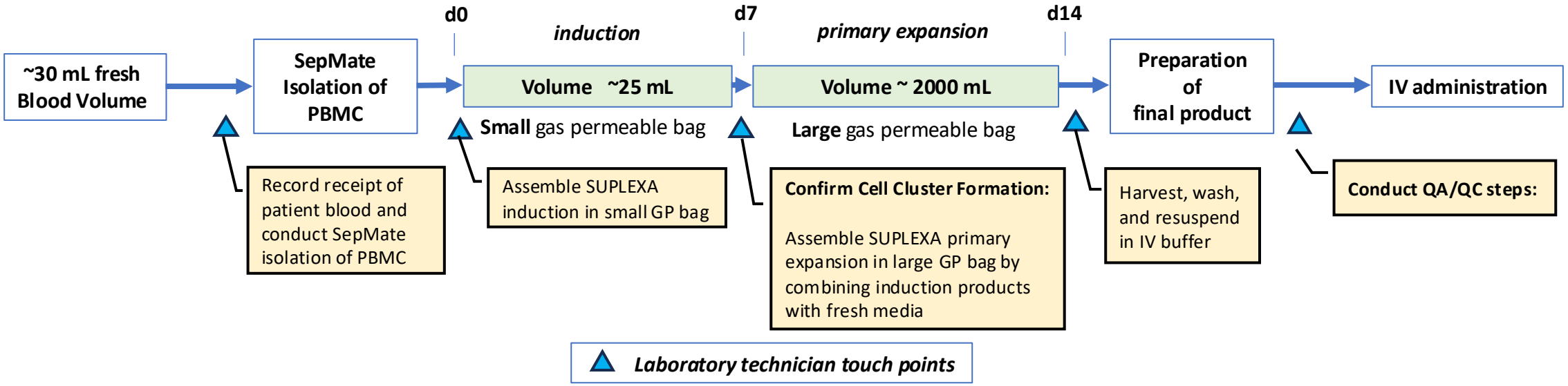
1. We show consistent ENLIST cell induced generation of SUPLEXA cells from CLL patients and CLL cells die or are killed during the SUPLEXA manufacturing process
2. CyTOF single-cell phenotyping of SUPLEXA cells from CLL patients and NHVs showed overlapping phenotypes, but also some important differences:
   - No TCRγδ T cell expansion from CLL PBMCs
   - Expansion of ILC-like cells in CLL patients – CD45+,CD39+,CD69+, Tbet+, GranzymeB and SLAMF6+ cells without T, B, myeloid, or NK markers
3. SUPLEXA cells from NHV and CLL patients showed identical levels of potent tumor cell killing activity and cytokine production profiles with normal distribution of heterogeneity

Funding: Alloplex Biotherapeutics, Inc and NCI R01CA258924 (PI Brown)

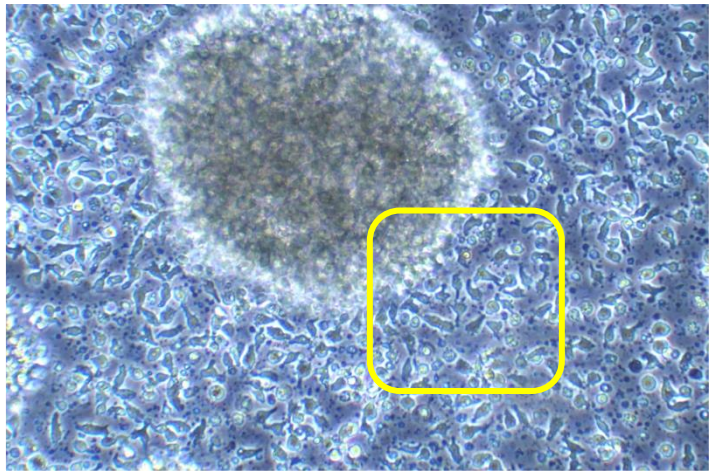
Note: This program was part of a collaboration with the DFCI.

Alloplex

Simplicity is key to Local Manufacturing



The stage is set for the clinical testing of SUPLEXA cells in hematologic malignancies



Please visit us at
www.alloplexbio.com
to learn more