IMMUNO-ONCOLOGY INSIGHTS

MODALITY/PLATFORM DEVELOPMENT TRENDS

SPOTLIGHT

COMMENTARY

Alloplex Biotherapeutic's SUPLEXA cells represent a new type of autologous adoptive cellular therapy for cancer

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SUPLEXA immunotherapeutic cells are derived by activation, differentiation, and expansion of cancer patient peripheral blood mononuclear cells (PBMCs) by an 'training' melanoma tumor cell line that has been engineered to express multiple immunomodulatory factors. SUPLEXA cells generated by our manufacturing process develop into potent and broadly cancer reactive cells that do not damage normal cells or tissues. Our SUPLEXA cellular therapeutic approach is currently being tested in a Phase 1 metastatic cancer clinical trial in Australia. The majority of the first 20 metastatic cancer patients, which had progressive disease (PD) have shown disease stabilization without any drug related adverse events. Furthermore, several patients have reported improved quality of life. In addition to tumor size measurements, we performed comprehensive, longitudinal single-cell PBMC profiling and plasma cytokine measurements of enrolled patients as a measure of changes in immune health over time. Patients with stable disease (SD) showed marked changes in specific immune cell type abundances and altered circulating cytokines that are indicative of improved immune health. These laboratory observations serve as pharmacodynamic markers of SUPLEXA activity, which will be used clinically to optimize the dosing schedule and select the target cancer patient population most likely to benefit. The lack of negative adverse event observations will facilitate the developmental path for SUPLEXA cellular therapy with feasibility to explore combinations with other cancer therapies without concern about compounding side effects. Our basic insights into the biology of SUPLEXA cells strongly suggest that our SUPLEXA cellular therapy approach is a novel and multivalent personalized cellular therapy with potential for treating multiple types of cancers.

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The cellular therapy space has witnessed an explosion of innovation beginning with CD19 targeted CAR-T cells for the treatment of leukemias and has been extensively reviewed [1–4]. These first-in-class autologous therapies involved the transduction of T cells obtained from a patient's peripheral blood with a lentiviral vector encoding a chimeric antigen receptor (CAR). These first-generation CARs encode a type I transmembrane proteins comprised of an extracellular scFv domain with antibody-like specificity to the CD19 antigen linked to a transmembrane segment and an intracellular signaling domain which activates T cells following CAR engagement with its cognate ligand. While the CD19 target protein is broadly expressed on acute leukemia cells, it is also found on normal B cells, which means that all B cells are also eliminated upon treatment with CAR-T therapeutic cells. Fortunately, it has been established that long term B cell depleting therapeutics such as the anti-CD19 mAb Rituxan® is well tolerated. These early efforts have led to the commercialization of three therapies in the CAR-T class, including Novartis/University of Pennsylvania (Kymriah®), Bristol Meyers Squibb (BMS)/Juno Therapeutics (Breyanzi®), Gilead/Kite (Yescarta®) and has initiated many follow-on CAR-T programs among various biotechnology companies using updated CAR design elements and technologies [3,5].

While these early CAR efforts focused on cells of the adaptive immune system, more recent efforts, have explored leveraging the arguably superior inherent features of innate immune cells to counter tumors by CAR engineering strategies in NK, iNKT, γδ T cells, macrophages and B cells [6–9]. In addition, induced pluripotent stem cells (iPSC) have presented an unparalleled opportunity which has been exploited to accommodate significantly more complex multi-step engineering approaches that extend beyond a single CAR [10–12]. For example, knocking out β-2 microglobulin to reduce HLA Class I expression to reduce host versus graft rejection of allogeneic immune cells [13,14]. iPSCs have the virtue of being infinitely expandable and able to support multiple genetic manipulations leading to the development of stable cell lines that can then be differentiated to the desired immune cell type just before administration to a patient. While it remains to be proven that iPSC derived immune cells are functionally equivalent to their naturally occurring counterparts, especially those mechanisms involving immune memory, they clearly can mediate anti-tumor activity. Cells of the innate immune system also avoid the requirement for HLA matching, which makes them better candidates for allogeneic cellular therapies.

Despite a demonstrable level of anti-tumor activity by cells produced by these varied approaches, solid tumors remain substantially recalcitrant to cytolysis via these approaches. One major hypothesis to account for this *in vivo* resistance is that the tumor microenvironment (TME) of various cancers is a major impediment to cellular therapeutics gaining access to the tumor [15–17]. Major efforts have been made to address modifying the tumor microenvironment to make it more susceptible to immunotherapy [18,19]. Furthermore, the TME is enriched by immunosuppressive cells such as Tregs, MDSC and M2 macrophages, all of which produce immunosuppressive cytokines like TGF-β and IL-10 that would suppress most immune cells even if they did gain access to the tumor. The problem is further compounded by the possibility that different solid tumor types may have different immunosuppressive mechanisms of action in their TME and elsewhere [20], which may impede a single, one cell type cellular therapy approach as well. Beyond the choice of effector immune cells to be deployed against cancer, the most limiting feature for any CAR based approach is the need for a specific target protein that distinguishes tumor cells from normal tissues. CD19 is an example of a tumor target that is shared with a dispensable normal cell type, but most tumor target antigens are expressed to some level on normal tissue and are responsible for some of the CAR-based therapeutic side effects.

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In contrast to the various CAR approaches listed above, tumor infiltrating lymphocytes (TILs) have offered a non-engineered autologous approach with the advantageous feature that they are derived from cells that have already demonstrated the capacity to enter and survive in the TME [21,22]. One must however question the hypothesis behind expanding a TIL population that was not able to control the tumor in the first instance. It has been demonstrated that such cells can be functionally impaired [23]. Beyond this theoretical consideration, perhaps the most limiting factor in TIL cell therapy development has been the manufacturing process. For example, since TILs are tumor derived, it is essential to demonstrate no tumor cells remain in the expanded final product since that would be tantamount to providing metastatic cells to the patient. In addition, Lovance, an early leader in the TIL field, has been considerably delayed with issues related to establishing a potency assay acceptable to the FDA and more recently Instil has had to suspend enrollment in its first clinical trial at great reputational cost because of inability to consistently make their cellular product. These difficulties have resulted in TILs having yet to yield a commercial product despite preceding CAR technology by several decades [21].

HISTORICAL CONTEXT

The extraordinary number of approaches being pursued by both academic and commercial groups have spanned the range of cell types (e.g., NK, iNK-T, T cells, macrophages) and sources (e.g., PBMC, cord blood, iPSC) (Table 1). What they have in common however is a focus on a single cell type with a specific engineering concept, be it CAR, genetic insert/deletion, or a combination. Alloplex Biotherapeutics Inc., hereafter referred to as Alloplex, reasoned that picking a single immune cell type or engineering approach was biased by limiting the cellular therapy to a specific antitumor response. Furthermore, we felt that a multifaceted cellular approach against tumor cells would provide a more robust antitumor response and that natural immunostimulatory mechanisms could generate tumor killing cell subsets without genetically engineered enhancements.

Accordingly, Alloplex has focused its efforts on a differentiated approach that was inspired by an earlier generation of cellular vaccine developers. Specifically, GVAX developed by Cell Genesys over 25 years ago utilizing a prostate tumor cell line (PC3) genetically engineered to express GM-CSF (GVAX), an immunomodulatory cytokine that is known to stimulate the maturation and function of dendritic cells (DCs), a type of professional antigen presenting cell [24,25]. The hypothesis driving this approach was that GVAX would be able to release cross-reactive prostate tumor antigens to DCs while simultaneously activating their function and in so doing, lead to a more productive anti-tumor immune response. Early-stage clinical trials demonstrated that GVAX could be used to yield clinical responses in prostate cancer patients. These early data were promising [26] but the limited durability was an impediment to further development, which led to perhaps the most consequential development decision made in the pivotal registration trials; namely to combine GVAX vaccination with chemotherapy [27]. Unexpectedly, the pivotal trials, VITAL-1 and VITAL-2, showed that patients treated with the combination of cellular vaccination and chemotherapy performed considerably worse than patients treated with chemotherapy alone with a shorter progression free survival (PFS) and overall survival OS. Unfortunately, no vaccination-only arm was included in these trials. The outcomes of the VITAL registration trials were deemed a failure with devastating consequences not only for the GVAX developers but also for the field of cellular vaccination approaches for immunotherapy leading to reluctancy in investor support for second generation efforts.

At Alloplex, we interpreted the data for the VITAL trials in a different and more favorable way. We reasoned that if vaccination was not having an effect, then there should

TABLE 1

Representative biotechnology companies pursuing diverse cell therapy strategies.

Therapeutic cell type	Company
Non-engineered trained immune cells	Alloplex Biotherapeutics
CAR-T cells (autologous and allogeneic)	Allogene
	Sana
	Poseida
	Precision Biosciences
	Adaptimmune
TILs (autologous)	Iovance
	Instil
iPSC (allogeneic)	FATE
	Century Therapeutics
	Cytovia Therapeutics
	Shoreline Biosciences
	BrightPath Biotherapeutics
NK cells (autologous and allogeneic)	Nkarta (allogeneic)
	NKGEN
iNKT (autologous and allogeneic)	MINK (allogeneic)
γδ T cells (autologous and allogeneic)	Adicet (allogeneic)
Macrophages (autologous)	Carisma Therapeutics
	Myeloid Therapeutics
B cells (autologous)	Be BioPharma

be no difference in PFS and OS between the two study arms. Instead, we suspect that the GVAX cellular vaccine is inducing an immune response in patients that is being negated by the combined chemotherapeutic treatment specifically eliminated key effector immune cells that were activated to proliferate in response to the vaccine. The elimination of these vaccine activated immune cells specifically hobbled the emerging anti-tumor response. Alloplex used this realization to conclude that a GVAX cellular vaccine approach may indeed induce a beneficial anti-tumor response. This led us to further speculate that if engineered expression of one immunomodulator on tumor cells could provide beneficial immune effects then perhaps it may be justified to test additional immunomodulators both individually and in combination to further augment this tumor vaccine strategy.

SUPLEXA ORIGIN STORY

Alloplex expanded on the seminal GVAX concept by exploring the combinatorial space of a highly curated list of immunomodulatory proteins selected for their ability role in activating complementary immune cell types. Higher order combinations were achieved by a reiterative process whereby multiple rounds of viral transductions were used to introduce immunomodulators into a tumor cell line with *in vitro* testing after each cycle. Using PBMC as the starting material (Figure 1), highly engineered tumor cells called ENLIST (engineered lymphocyte stimulator) cells were tested using *in vitro* mixed lymphocyte tumor reaction (MLTR) assays. The MLTR allowed us to precisely evaluate the biological effects of ENLIST cells on PBMCs by measuring immune cell activation, differentiation, proliferation, cytokine production, and most importantly, cytolytic activity.

We observed that sequential addition of immunomodulators in the ENLIST cells greatly affected PBMC proliferation, cytokine release, and tumor cytolytic activity of the expanded PBMC populations. Our engineering efforts were halted only when we reached the maximum value in the dynamic range of the assays employed. One

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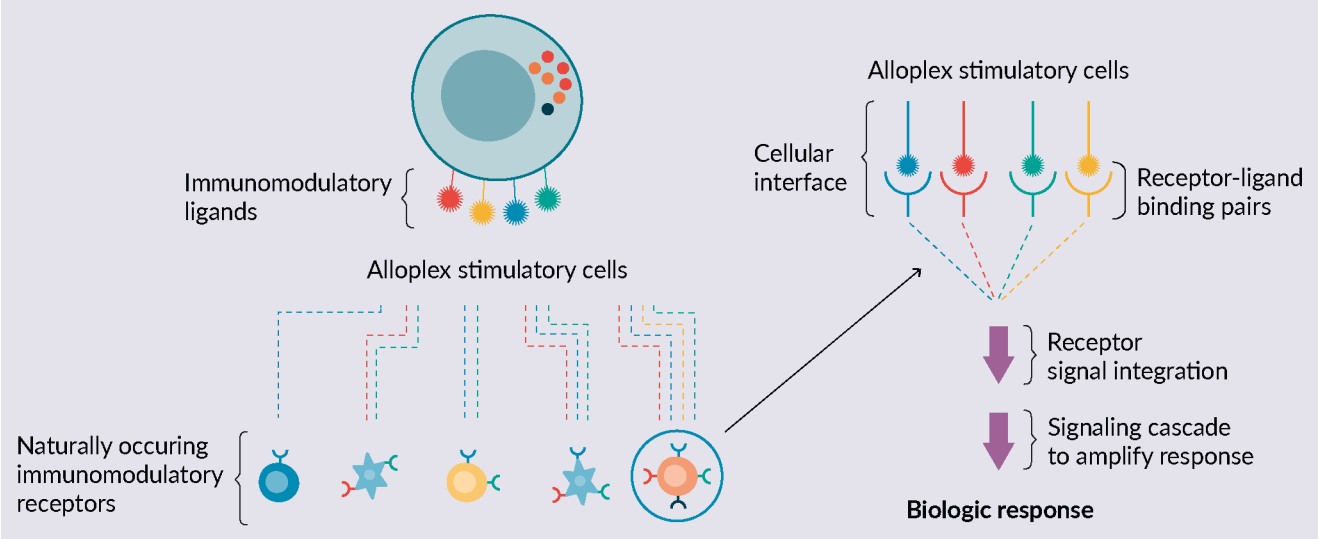
observation of particular concern was a dramatic increase in the inflammatory cytokines released during the MLTR, which we felt might lead to serious adverse events in a cellular vaccine setting. However, we were fortunate that the MLTR approach showed us that *ex vivo* cellular expansion was possible and could be used as basis for a manufacturing process leading to an autologous or allogeneic adoptive cellular therapy for cancer. We called these *ex vivo* expanded cells SUPLEXA therapeutic cells – alluding to the multiple immunomodulators used to activate complementary immune cell types. Incidentally, the name SUPLEXA derives from the word 'suplex', which is a technical term in wrestling to describe an offensive maneuver intended to control the opponent.

During the construction of ENLIST cells, numerous combinations were tested but one set of immunomodulators proved to demonstrate a remarkable and unexpected synergistic activity and is the subject of an issued US patent (US10731128B2). This core set is comprised of a CD28 ligands (CD80 or CD86), OX-40 ligand and CD27 ligand. Each of these ligands showed little individual enhancement of PBMC activity in the MLTR, but when used together, a striking 300-fold induction in the number of CD8 positive, cytotoxic T cells occurs. Indeed, this unexpected synergy demonstrates that this approach for analyzing the combinatorial space of immunomodulators can be utilized to efficiently identify previously unappreciated functionally intersecting or synergistic immune pathways. Using this initial core set as a starting point, Alloplex significantly expanded and refined immunomodulators and moved into a higher order of combinatorial variations. The final combination of immunomodulators used in ENLIST cells and specific cell manufacturing know-how are foundational to Alloplex initiatives in cellular therapy and are proprietary trade secrets. We now refer to ENLIST cells as immune training cells because they have the capacity to train PBMCs to develop into immune cells with potent anti-tumor effector function and phenotype.

Most impressively, SUPLEXA cells demonstrated broad cytolytic activity against all tumor cell lines tested, irrespective of HLA matching or tumor type. This HLA

FIGURE 1

The Alloplex approach.



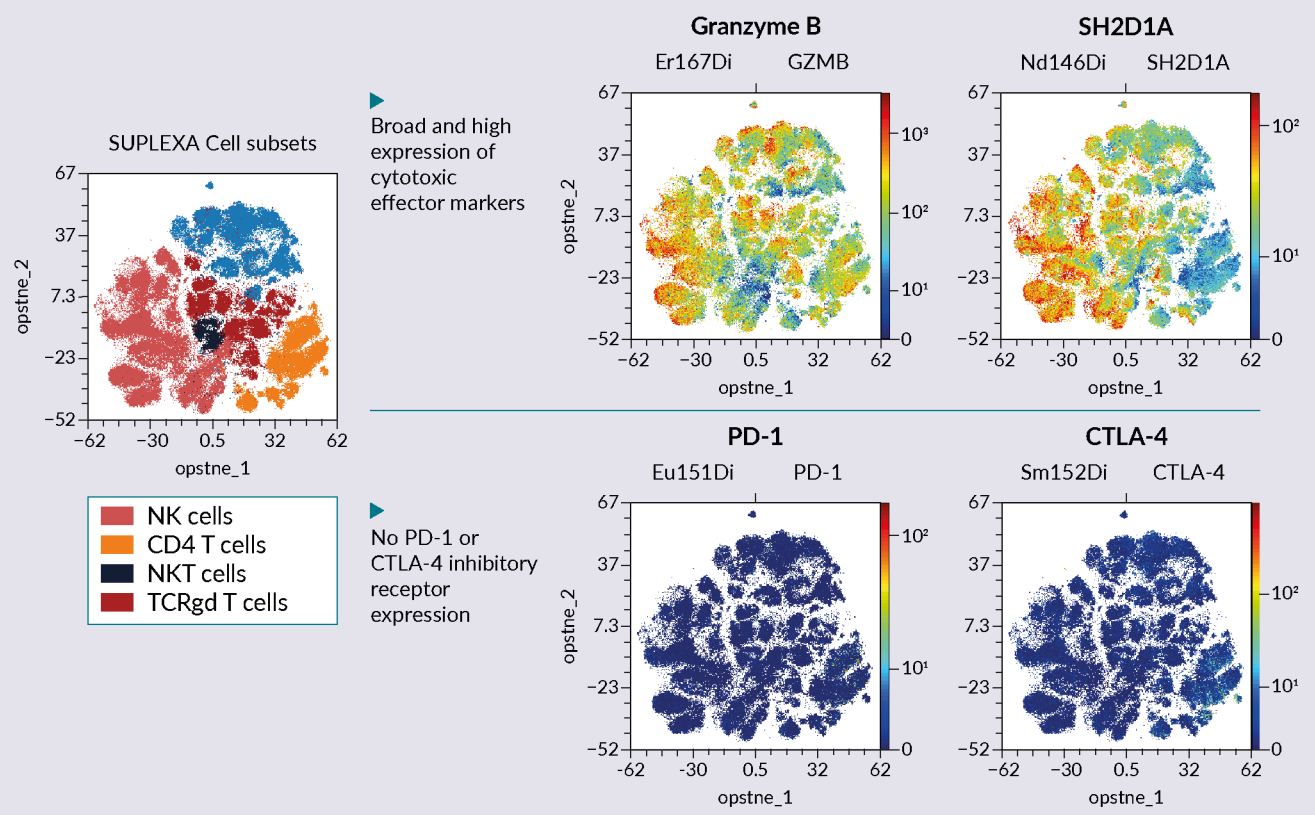
Engineered lymphocyte stimulator (ENLIST) training cells are engineered to express a combination of immunomodulatory ligands designed to stimulate a set of complementary immune cells to acquire tumor killing capacity. These activated immune cells are cytolytic against a broad array of tumor cells but without causing harm to normal cells. It is hypothesized that activation through multiple signaling pathways is integrated and amplified to yield a biologic response such as differentiation, proliferation, and cytokine production.

independence is perhaps not surprising given the large percentage of innate immune cell types such as NK, NK T and γδ T cells that express the NKG2D activation marker, which is known to bind stress signals typically found on the surface of cells undergoing metabolic stress due to infection, cancerous transformation, or senescence. Comprehensive phenotyping by mass cytometry (CyTOF) identified the cellular composition of SUPLEXA cells as a mixture of NK cells, $CD8^+$ and $CD4^+$ T cells, TCR γδ T cells, and NK T cells. Phenotyping by CyTOF showed that SUPLEXA cells express high levels of tumor cytolytic markers like granzyme B and SH2D1A, but not inhibitory checkpoint inhibitors like PD-1 or CTLA-4 (Figure 2). We have performed iRepertoire (Huntsville, AL, USA) T cell receptor (TCR) sequencing analysis of αβ and γδ TCRs in SUPLEXA cells and identified a significant increase in αβ and γδ TCR clonality to suggesting that there may be antigen-specific T cell activation and expansion occurring during SUPLEXA manufacturing. Future work will seek to identify antigen specific mechanisms involved in T cell training by ENLIST cells.

While genetic engineering of the SUPLEXA therapeutic cells was also considered, we found that the broad anti-tumor activity of SUPLEXA cells we reasoned that further genetic engineer of SUPLEXA cells was not necessary. Avoiding genetic engineering allows for a more efficient and less expensive manufacturing process than CAR-T processes. A first-generation SUPLEXA manufacturing process has already been developed for our Phase 1 clinical trial (Figure 3). SUPLEXA cells are autologous and therefore have lower risk for adverse events or clinical complications like graft-versus-host (GVH) disease or host-versus-graft (HVG) rejection

FIGURE 2

CyTOF analysis of SUPLEXA cells.



A composite CyTOF analysis of SUPLEXA therapeutic cells from the first three patients at the end of the expansion process reveals high expression of the cytotoxic effector markers, Granzyme B and SH2D1A and no expression of checkpoint receptors PD-1 and CTLA4.

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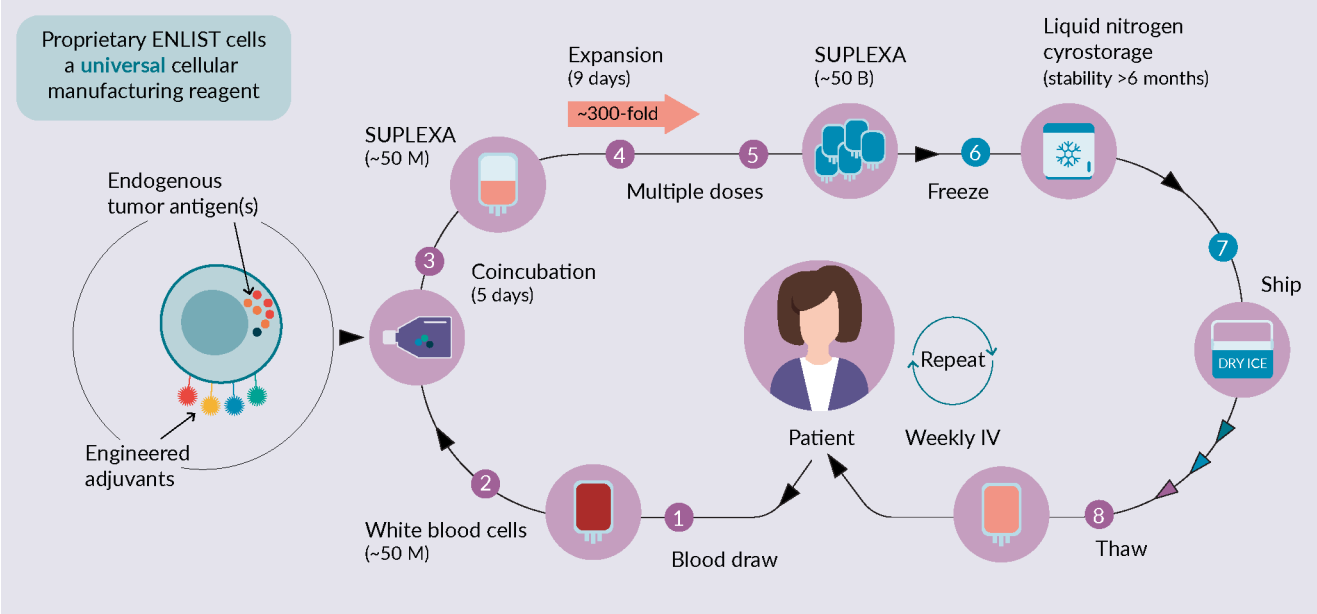
FIGURE 3

The SUPLEXA manufacturing process.



The multi-step manufacturing process begins with 1) a blood draw of about 50 mL, followed by 2) isolation of PBMC using Ficoll density centrifugation, 3) coincubation of PBMC with ENLIST training cells to induce SUPLEXA cells, 4) expansion of SUPLEXA cells with cytokine support by approximately 300-fold, 5) aliquots of SUPLEXA cells into individual IV dosage bags, 6) controlled freezing followed by long-term cryogenic storage in liquid nitrogen, 7) shipping to the clinical site on dry ice and 8) controlled thawing followed by IV administration back to the patient.

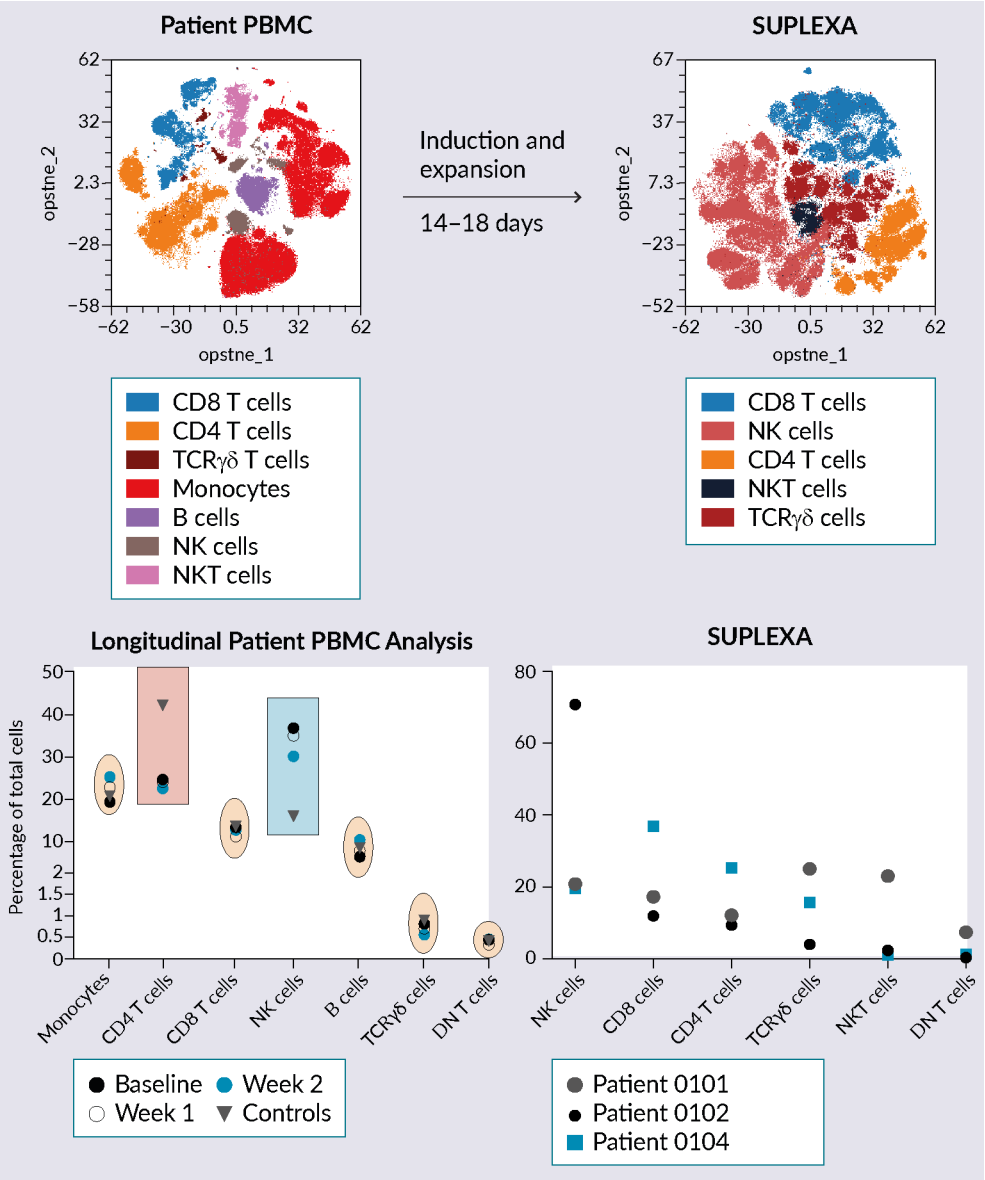
of SUPLEXA cells as compared to allogeneic cellular therapeutics.

Furthermore, by virtue of not being engineered, SUPLEXA cells do not carry the theoretical risks associated with possible tumorigenesis resulting from the lentiviral transformation. SUPLEXA cells develop from the patients' PBMCs by activating naturally occurring receptors functioning at regulated normal physiologic levels. We posit that their anti-tumor activities will also be naturally physiologic when given as a therapeutic cell. By contrast, CAR proteins deliver supraphysiologic signals that can lead to the clinical toxicities such as cytokine release and tumor lysis syndromes. The SUPLEXA therapeutic cells currently in clinical testing are a mixture of cells comprised of NK, NKT and T cells but are notably devoid of Tregs, myeloid and B cells. The exact composition of SUPLEXA therapeutic cells varies among individuals owing to the autologous and personalized approach and the nature of PBMCs from cancer patients that have received diverse prior therapies (Figure 4). Despite a significant level of inter-patient variability in percentage immune cell types, SUPLEXA cells consistently show similar activation morphology, functional phenotypic marker expression, and consistent cytolytic potency profiles. These phenotypes of SUPLEXA are used as measures for our quality control release assay (Figure 5).

By contrasting SUPLEXA cells against CAR-T cells highlights several additional key differences (Figure 6) with implications for clinical trial design and use as a broadly active cancer therapy. It was anticipated for instance that unlike CAR-T cells, SUPLEXA would not induce cytokine storms or work so vigorously as to induce tumor lysis syndrome. Implementation of SUPLEXA in a clinical setting would also prove very different from that of CAR engineered cells because chemotherapeutic preconditioning and systemic IL-2 treatments are often used to foster *in vivo* expansion of a single dose of about 250 million CAR engineered cells [28]. Multiple lines of evidence support the rationale for the use of chemo preconditioning [19,29] but some evidence is

FIGURE 4

CyTOF analysis of PBMC and SUPLEXA therapeutic cells from the first three patients.



DN T cells: Double-negative T cell; NK: Natural killer cells; NKT: Natural killer T cell; PMBC: peripheral blood mononuclear cell;: TCRγδ: T cell receptor gamma delta.
Compared to normal controls, the PBMC analysis of the first 3 cancer patients revealed a lower percentage of CD4⁺ cells both at baseline and over the initial 2 weeks of SUPLEXA cell therapy but an increase in the percentage of NK cells. SUPLEXA cells showed considerable variability in cell composition between patients as anticipated due to individual heterogeneity in patients and the personalized therapeutic approach.

also emerging, which suggests that it may not be necessary [30]. This issue is critical because the chemo preconditioning used with CAR engineered cells comes with significant toxicities, including profound cytopenia. Immune system recovery after cell ablation is a dynamic process that can span years and often does not recover to pre-treatment cellular composition [31]. Since SUPLEXA cells can be expanded to large numbers *ex vivo* without loss of activity and may not require *in vivo* expansion, they can be administered to the patient in ten-fold larger cellular doses than CAR cell therapies. Hence the patient will not experience preconditioning induced neutropenia, lymphopenia, thrombocytopenia. Furthermore, the ability to

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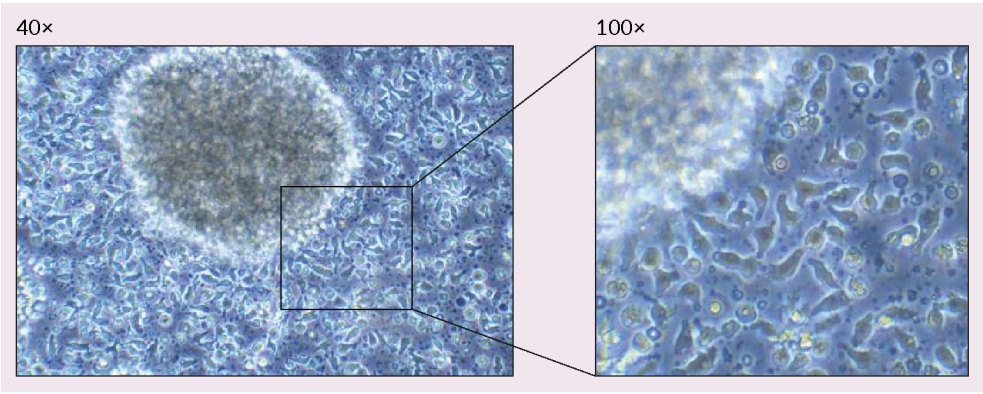
FIGURE 5

Photomicrographs of SUPLEXA cells.



SUPLEXA cells grow in dense clusters and develop elongated forms as shown in the low and high magnified photomicrographs of SUPLEXA cells in culture at 2 weeks after induction and expansion. The morphology of SUPLEXA cells is typical of activated lymphocytes with large oblong polarized cells.

manufacture unlimited numbers of SUPLEXA therapeutic cells allows for the ability to administer multiple doses, something that would not be possible if chemotherapeutic preconditioning were required prior to every dose.

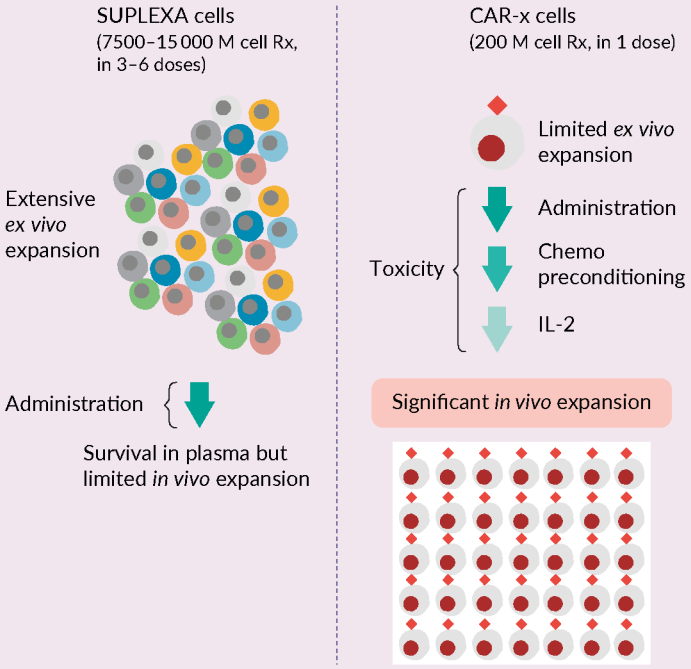
For our initial clinical trial, we adopted a once weekly dosing regimen of 2.5 billion cells for a minimum of 3 weekly doses and this dosing schedule is limited only by the manufacturing yield of the patient's specific

FIGURE 6

Differentiation of SUPLEXA cell from CAR-x cell therapies.

SUPLEXA versus CAR

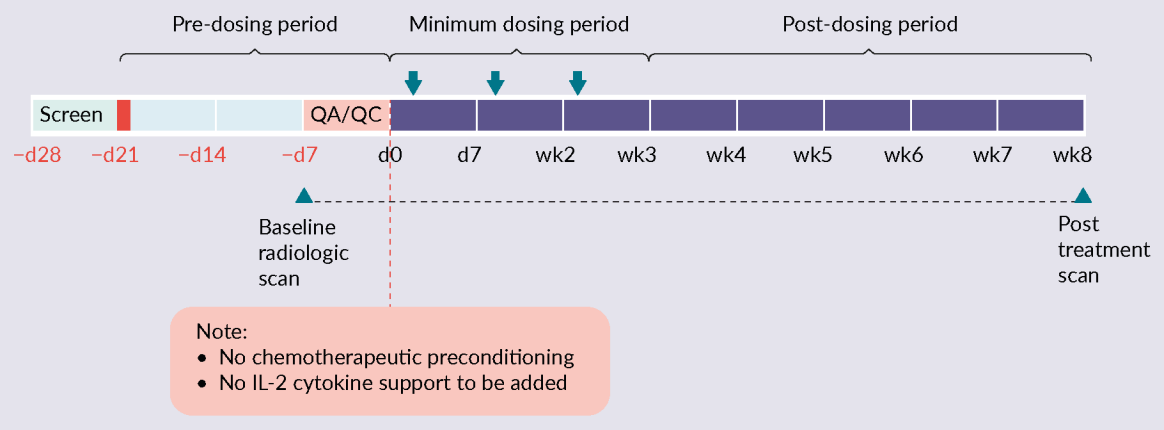
Feature	SUPLEXA	CAR-x
Overall	Non-engineered Hetreogeneity high	Engineered Hetreogeneity limited
Spectrum of activity	Broad pan-tumor	Tumor-type specific
Growth factors needed for expansion	Exogenous only	Exogenous and intrinsic due to CAR signaling domains
Expansion potential	High	High
Administered dose	7500–15 000 M cells	200 M cells max. (Yescarta)
Chemo pre-conditioning	NO No need for additional survival factors or cell expansion	YES Induce homeostatic cytokines and reduce cytokine sinks for greater therapeutic cell expansion



SUPLEXA therapeutic cells are differentiated from CAR modified (CAR-x) cell therapies as listed in the table. Unlike CAR-x cells which are administered in a single dose of 250 M cells, SUPLEXA are administered as multiple weekly doses of 2.5 B cells or 30–60 times as many cells. As such *in vivo* expansion is not essential for SUPLEXA cells and thus does not require chemotherapeutic preconditioning to foster such expansion.

FIGURE 7

SUPLEXA-101 study design.



This is a single agent basket trial designed to enroll metastatic solid tumor patients or those with hematologic malignancy. A screening period 21–28 days prior to first dose confirms patients meet enrollment criteria. Blood is then drawn for SUPLEXA manufacturing approximately 3 weeks prior to the first SUPLEXA dose. The 2-week manufacturing period is shown in blue followed by about 1 week of quality control prior to product release. A minimum of three weekly SUPLEXA doses comprised of 2.5 billion cells is shown although more is possible depending on the manufacturing yield. Importantly, no chemo preconditioning or IL-2 cytokine support is used which spares the patient significant toxicity. Scans are performed at baseline and approximately every 8 weeks afterwards. The focus of this Phase 1 study is safety but has been used to demonstrate single agent activity and for exploratory analyses.

SUPLEXA batch. Having observed no drug related adverse events in the first 20 patients, we are now in the position to explore more intensive multiple dosing regimens and the utility of combining SUPLEXA treatments with already approved anti-tumor drugs such as Rituxan, Herceptin® and checkpoint inhibitor antibodies.

MANUFACTURING PROCESS

The first generation SUPLEXA manufacturing process currently requires approximately 2 weeks of laboratory expansion followed by 1 week each for QA/QC and logistical coordination of delivering the first dose back to the patient. The 4 weeks that patients must wait for their individualized therapy

TABLE 2

Early clinical findings on the first 11 patients.

Patient	Cancer type	RECIST	
0101	Anal canal squamous cell carcinoma	SD	
0102	Ovarian (serous papillary)	SD	
0104	Ovarian		PD
0105	Endometrioid carcinoma		PD
0106	Cervical SCC	SD	
0107	Pancreatic	SD	
0201	Ureteric transitional cell carcinoma		PD
0202	Endometrioid carcinoma		PD
0203	Endometrioid carcinoma of the ovary	SD	
0204	High-grade serous carcinoma of ovary		PD
0205	Bladder transitional cell carcinoma	SD	

The first 11 patients in the SUPLEXA phase 1 study had progressive metastatic disease from diverse tumor types upon enrollment. Each patient received a minimum of 3 weekly SUPLEXA doses comprised of 2.5 billion cells per dose without any reported drug related adverse events. RECIST analysis resulting from imaging approximately 8 weeks after first SUPLEXA dose revealed disease stabilization in a major of the patients (6/11) with the 5 patients showing progressive disease comprised of ovarian or uterine disease, all of which had ascites at the time of enrollment.

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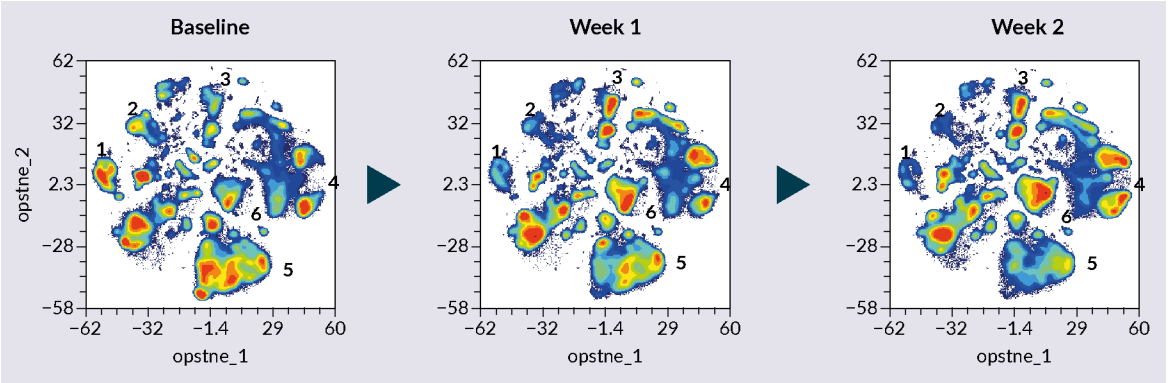
FIGURE 8

CyTOF analysis of the PBMC from the first three patients (0101, 0102 and 0104) over the first 2 weeks of therapy demonstrated favorable changes in the immune cell profile.



Several examples of undesirable cell populations decreasing over time are indicated by number 1 (exhausted CD4⁺ T cells), 2 (exhausted CD8⁺ T cells) and 5, CD16⁺/Arginase-1+ suppressive monocytes. Several examples of desirable cell populations increasing over time are indicated by number 3 (cytotoxic NK-T cells), 4 (activated proliferating monocytes) and 6 (B cells).

is perhaps the biggest current liability of the approach; and one which can lead to anxiety for the patient. In the future, we hope to bridge this waiting period for the patient by using a single dose of allogeneic SUPLEXA cells immediately after drawing blood from the patient.

SUPLEXA cells exhibit individual batch-to-batch variability in immune cell lineages (e.g., NK versus T cell ratio). However, they consistently express an activation signature that is a composite of cytolytic cells (Granzyme A, B, Perforin and Granulysin) and cells that have uniquely acquired an antigen presenting cell like phenotype (HLA Class II, IL-3R, CD28 ligands). Given this observation, we suspect that SUPLEXA cells may be able to present released tumor antigen after the initial cytolysis of a tumor target and thereby activate and amplify the response of host immune cells against the tumor. However, this is the subject of ongoing research at Alloplex that is addressing the biology of SUPLEXA cells. Since these are unique and consistent findings, these activation markers comprise the foundation for a phenotypic release assay conducted on each SUPLEXA batch. Complementary to the phenotypic analysis, each SUPLEXA batch undergoes assessment in a cytolysis assay in which a tumor cell line is employed as a reference target to ensure a minimum level of anti-tumor activity is reached in each SUPLEXA batch.

FIRST SUPLEXA CLINICAL TRIAL

SUPLEXA cells are currently being tested in a Phase I basket trial designed to enroll patients with solid tumors and hematologic malignancies (clinicaltrials.gov, NCT05237206). The trial is enrolling highly pre-treated patients who have progressed through numerous prior therapies and therefore have no standard of care treatment options left. The trail design is shown in (Figure 7). This trial makes use of a first-generation manufacturing open process that begins with about 50 mL of whole blood. PBMC are isolated using standard density centrifugation isolation in a regional manufacturing facility, are activated to differentiate by proliferation attenuated ENLIST cells, and then expanded to yield a minimum of 7.5 billion SUPLEXA cells for administration in 3 or more IV doses of 2.5 billion cells each. The numbers of SUPLEXA doses have varied among enrolled patients (ranging from 3 to 15 doses), which was anticipated given the autologous nature of the starting PBMC material. Despite this

FIGURE 9

Plasma from the first three patients were screened for cytokine levels by 40 cytokine Luminex assays.

The 40 cytokine Luminex panel used to measure plasma cytokine levels included Luminex bead regions to detect the following cytokines: IL-2,TNFα,IL-4,IL-18,IL-1α,IL-1β,IL-1RA,IL-5,IL-10,IL-33,IL-23,IL-22,IL-6,IL-21,IL-8,Tweak,IFNβ,MCP-1,G-CSF,MIP-1α,IFNγ,ST2,GM-CSF,IL-13,Trem-1,MIP-3α,GROα,Rantes,IL-17A,ENA-78,P-DGF-AA,PDGF-BB,MCP-3,MIG,MDC,FLT3L,IL-15,IP-10,TGFβ1, and MCP-2. Inflammatory and immune suppressed phenotypes were detected in these patients and SUPLEXA treatments had effects on systemic cytokine levels in each patient as illustrated in these radar plots. Patient 0101 had high levels of cytokines, while patients 0102 and 0104 had low levels of cytokines. All patients showed increases in IL-8, Rantes, and FLT3L.

intrinsic variability, SUPLEXA cells reproducibly demonstrate phenotypic and cytolytic release assays within acceptable ranges to be used in patients.

The emerging data from the first 11 patients receiving a minimum of 3 doses demonstrates disease stabilization in most patients as determined by the first post treatment imaging time point taken at ~8 weeks post first SUPLEXA infusion (Table 2). Remarkably, this was achieved with no reported drug related adverse events, not even infusion site reactions. The only feature on which patients have remarked is a garlic or sweet corn odor of limited duration, which is likely due to the DMSO in the cryopreservation media. In addition to these early safety and efficacy findings, we are highly encouraged by anecdotal accounts from the nursing staff and patient reports that suggest an overall improved quality of life with instances of increased energy and reduced pain and narcotic use. The trial remains open to enrollment and patients continue to be monitored.

As part of our exploratory studies, patient blood samples were collected over the course of the study and assessed for cellular composition (Figure 8) and plasma inflammatory markers (Figure 9). Surprisingly the first several patients showed an improvement in 'immune health' based on a comprehensive CyTOF phenotypic analysis of longitudinal PBMCs and Luminex cytokine profiling of plasma samples. Interestingly, we found that a patient with high levels of systemic cytokines showed a progressive reduction in circulating cytokine levels, which suggests that SUPLEXA therapy may have significant impact on the pro-inflammatory nature of certain types of cancer (Figure 10). These pharmacodynamic observations provide an unexpectedly important tool for optimizing the SUPLEXA cell dosing regimen and target population. Moreover, these pharmacodynamic measures on overall immune health are especially important for autologous SUPLEXA cell treatment since pharmacokinetic measures are not possible owing to the difficulty of distinguishing SUPLEXA cells

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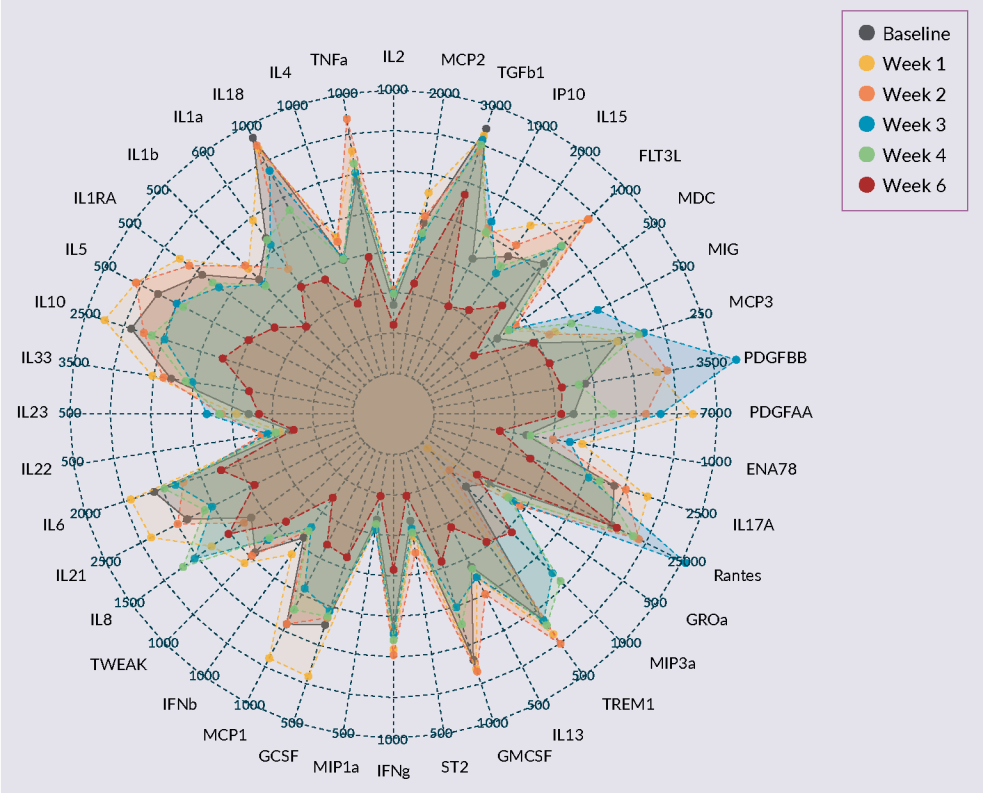
FIGURE 10

In a patient with high baseline levels of inflammatory cytokines, SUPLEXA cell therapy reduced circulating cytokine levels.



Patient 0101, 56- year-old male with metastatic squamous cell carcinoma presented at baseline with uniformly elevated cytokine levels as depicted on this radar plot. This patient received 5 weekly doses of SUPLEXA cell therapy of 2.5 B cells. Shown are progressively decreased inflammatory cytokines at week 1, 2, 3, 4 and 6 weeks following the first dose. Reductions in TNF, IL-6, and IL-1/IL-18 levels are consistent with improved immune health.

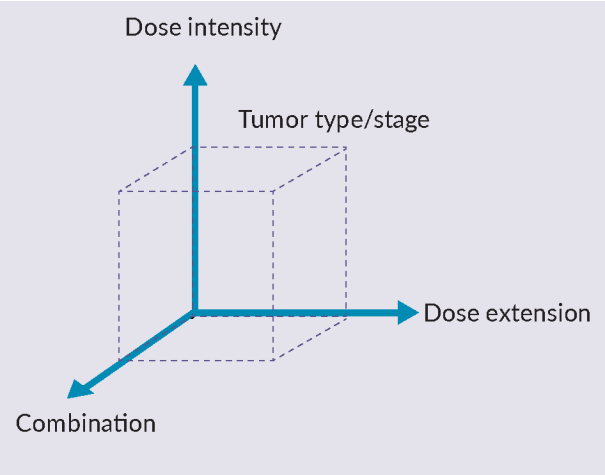
from normal activated cells endogenous to the host. Fortunately, the pristine safety profile exhibited by SUPLEXA so far creates an unprecedented opportunity to consider combination therapies with tumor targeting antibodies as well checkpoint inhibitors/engagers to enhance the efficacy of this autologous cellular therapy.

SUPLEXA cells are a developmental work in progress with many potential applications both in oncology, autoimmunity, infectious diseases, and senescence that will play out over the next 5–10 years (Figure 11). However, near term goals for the ongoing SUPLEXA trial include:

1. Optimizing the clinical SUPLEXA dosing regimen;
2. Exploring a second PBMC-derived SUPLEXA batch for responding patients;
3. Determining which tumor types are most likely to respond;
4. Correlating clinical responses with the pharmacodynamic assessments of immune health;
5. Incorporating validated quality of life measures into our clinical trials, which can capture clinical activity beyond what is apparent in scans and other laboratory assessments;
6. Integrating a single allogeneic SUPLEXA dose to cover the period in which the autologous SUPLEXA cells are being prepared.

FIGURE 11

Diagram of future development for SUPLEXA.



Future therapeutic decision for SUPLEXA will focus on several variables including tumor indication, dose schedule (number and intensity), potential for combination with other therapies, and industrializing the manufacturing process.

CONCLUSION

The Alloplex approach of activating and expanding immune cells by using a highly engineered training cell line represents a novel cellular therapeutic development platform approach with extraordinary promise fueled by our early emerging clinical safety and efficacy data. While the first generation SUPLEXA cells used an unbiased approach resulting in a final product comprised of multiple cell types, we have already demonstrated that the ENLIST immune training platform can be used to specifically enhance B cells, γδ T cells, and Treg cells. In the future, cells enriched for these specific cell types using this approach may have additional specific applications in infectious diseases and autoimmune indications. The pristine safety profile exhibited so far will undoubtedly facilitate the development of SUPLEXA cells either as a stand-alone therapeutic option or in combination with various biologic cancer therapeutic agents such as tumor targeting antibodies, checkpoint inhibitors and cell engagers. While initial SUPLEXA development has focused on an autologous approach, the integration of allogeneic approaches may also be possible in settings where shorter term bridging therapies are required until the patient's own cells can be prepared [32,33]. The enhanced second-generation manufacturing methods will also greatly facilitate future developments by increasing process efficiencies and exploiting economies of scale, critical features for making such therapies more accessible to patients.

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AUTHORSHIP & CONFLICT OF INTEREST

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