



Challenges and potential solutions in cellular immunotherapy

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COMPLEXITIES IN APPLICATION

It would not be an overstatement to say that in recent years cellular immunotherapies have demonstrated great therapeutic success in some cancers. However, several technical difficulties remain that prevent this field from achieving the large-scale success it originally promised, and this is largely the reason why chimeric antigen receptor T cell (CAR-T) has yet to break out beyond the confines of hematological malignancies. In this short paper, we briefly highlight some of the key challenges hindering the application of cellular immunotherapy for cancer treatment, and the strategies being employed to address them.

The playing field

As described in our paper "[An Analysis of the Cellular Immunotherapy Landscape for Cancer](#)", there are eight main types of cellular immunotherapy:

- **CAR-T cell** therapy involves genetically modifying T cells to express a CAR.
- **T cell receptor (TCR) therapy** utilizes the T cells' natural mechanisms to recognize antigens.
- Natural killer (NK) cells can be modified into **CAR-NK therapies** and used to target malignant cells.
- **Gamma-delta T cells ($\gamma\delta$ -T cells)** are defined by expression of heterodimeric T-cell receptors (TCRs) composed of γ and δ chains.
- **Tumor-infiltrating lymphocytes (TIL)** predate CAR-T therapies. They utilize T cells that already recognize and target a patient's tumor as a treatment for their cancer.

- **Cytokine induced killer (CIK) cells** are a subset of polyclonal T-effector cells possessing both NK and T cell properties.
- **Macrophages** are cells of the innate immune system that act as both phagocytes and antigen-presenting cells (APC). CAR-macrophages can be developed as cancer immunotherapies.
- **Dendritic cells (DC)** play a crucial role in immunosurveillance and are powerful APCs for the induction of antigen specific T cell responses.

Potency and persistence

The current generation of CAR-T cell therapies are somewhat limited in their degree of clinical benefit, especially outside the hematological setting, due to a lack of potency and persistence. But it seems that certain T cell characteristics can be exploited to possibly improve this. For example, Poseida Therapeutics is developing an anti-B-cell maturation antigen (BCMA) CAR-T cell therapy that is composed of long-lived, multipotent T memory stem cells (T_{msc}).^{*} This is essentially a young subset of T cells that are self-renewing, with the ability to survive for decades, and potentially for entire lifespans, [1]. Another interesting approach is one being pursued by City of Hope and National Cancer Institute (NCI) in which their CAR-T cell therapy is based on T central memory (T_{cm})-enriched CD8+ T cells; these are more persistent and are better at migrating to secondary lymphoid tissues than standard T cells [2].

^{*} At the time of publication (August 19, 2020), it has just been announced that Poseida's lead program in prostate cancer has been put on clinical hold by the FDA following the unexpected death of one of the patients 10 days after treatment. This further highlights the challenges inherent in bringing new technologies into the clinical setting.

Adverse effects

One of the key issues with certain cellular immunotherapies is the risk of adverse effects. For example, CAR-T cell therapy is still associated with cytokine release syndrome (CRS), encephalopathy syndrome and tumor lysis syndrome (TLS) [3]. This is largely due to complications in controlling the activation and proliferation of CAR-T cells once they have been administered, which leads to an over-active immune response [4]. There are however a few safety strategies in development to address this issue, some of which are listed in Table 1.

Some CAR-Ts can be regulated with specific agents, for example, Juno Therapeutics is developing a CAR-T cell therapy which contains a truncated form of epidermal growth factor (EGFR) [5]–[7]. By delivering the EGFR inhibitor cetuximab, these CAR-T cells can be cleared [8]. Bellicum Pharmaceuticals is developing GoCAR-Ts with an inducible MyD88/CD40 suicide switch, allowing the therapeutic effect to be modulated with the use of rimiducid [9]. Similarly, Autolus is developing CAR-Ts for solid tumors that contain the suicide gene rapaCasp9, which can be regulated with rapamycin [10].

Table 1: Safety mechanisms being utilized by pipeline CAR-T cell therapies (data from Clarivate’s Cortellis - accessed H1 2020). TCR, T cell receptor; GvHD, graft versus host disease; GM-CSF, granulocyte-macrophage colony stimulating factor; $\gamma\delta$ -T cell receptor, gamma-delta-T cell receptor; TRAP, intraductal microcatheter technology for transpapillary delivery.

Safety mechanism	# of pipeline therapies
Genetic editing to remove TCR to avoid GvHD	4 ¹
Safety/Kill switch	34
Tumor antigen specific binding	9
Conditional activation	5
Reversible therapy	3
GM-CSF knockout	3
IL6 knockdown/knockout	1
Full CAR-T cell activation requires activation of $\gamma\delta$ -T cell receptor	1
TRAP system	1
Predictable half-life	1
Lower affinity for target	1

Allogeneic CAR-T cell therapies come with at least the theoretical risk of graft versus host disease (GvHD) and so some researchers are using gene editing to eliminate receptors which mediate GvHD. For example, CRISPR Therapeutics’ allogeneic anti-BCMA CAR-T cell therapy uses CRISPR/Cas9 gene editing to remove the T cell receptor (TCR) and major histocompatibility complex 1 (MHC1) in order to escape GvHD and increase durability of the therapy [11].

TCR therapy can also lead to potential adverse effects and so similar mechanisms are being developed to mitigate these, such as Bellicum Pharmaceuticals’ CaspaCIDE safety switch technology which is modulated using the activator agents rimiducid or temsirolimus. If a patient experiences a serious side effect, these agents can be used to trigger apoptosis of the T cells and attenuation of the therapy [12]. Safety switches are also being built into CAR-NK cell therapies, for example, Takeda’s Tak-007 includes iCasp9 which can be modulated with rimiducid, leading to apoptosis of the CAR-NK cells if necessary [13].

However, one of the inherent issues with the use of kill switches is the most appropriate timing for activation. In practice, even severe CRS can be managed well in the clinic, and so physicians are reluctant to prematurely use the switch. By the time they decide to do so, it may be too late for the switch to take effect and benefit the patient.

Complexity of tumor targets

Yescarta, Kymriah and many pipeline cellular immunotherapies target CD-19 which is expressed mostly on B-cells; limiting the scope of these therapies beyond B-cell malignancies. While solid tumors present a much larger unmet need in terms of number of patients compared with hematological neoplasms, creating a CAR-T cell therapy that can actually successfully target solid tumors is notoriously difficult [8]. Many targets specific to solid tumors are often tumor-associated antigens (TAA) which have low levels of expression in normal tissues, meaning that

on-target off-tumor toxicity is a higher possibility [8]. Of course, CD19 is expressed on normal B cells which therefore are destroyed with CAR-T cell therapy, however, uniquely this can be clinically managed using life-long intravenous immunoglobulin (IVIg).

Multi-target CAR-Ts might be one way to improve solid tumor-targeting as they afford the opportunity for more specificity in cell targeting. Of course, the main benefit of bi-specific and multi-targeted CAR-T cell therapies is that they can prevent tumor escape through tumor plasticity, thereby reducing the likelihood of resistance to therapy. One such example is Aleta Bio's multi-targeted CAR-T cell therapy; a fusion protein comprising a CD19 extracellular domain and an anti-tumor antigen binding domain [14]. The technology was designed to address the critical issues of CAR-T persistence, tumor antigen loss and tumor antigen heterogeneity.

Immunosuppressive tumor micro-environment

Certain tumors, especially solid tumors, are 'immunotherapy-cold' i.e. they have an immunosuppressive tumor micro-environment [15]. This issue is currently being addressed in different ways, including combining CAR-T cells with pro-inflammatory cytokines. For example, Juno Therapeutics is developing an 'armored' CAR-T cell therapy that expresses IL-12 which has demonstrated enhanced proliferation, decreased apoptosis and increased cytotoxicity in the presence of immunosuppressive ascites [16].

TCR T cells also struggle to function effectively in certain immunosuppressive tumor microenvironments. One aspect of this tumor-elicited suppression is the interaction between programmed death-ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1), which causes T cell exhaustion [17]. Memorial Sloan Kettering Cancer Center (SKCC) is addressing this by programming CAR-T cells to secrete PD-1-blocking single-chain variable fragments (scFv). These scFv-secreting CAR-T cells improved the anti-tumor activity of

CAR-T cells and bystander tumor-specific T cells in mouse models of PD-L1+ hematologic and solid tumors, at levels similar to those seen from combination of CAR-T cells with a checkpoint inhibitor [18].

Technology specific challenges

CAR-NK cell therapy

NK cells do not persist after adoptive transfer without cytokine support, and so one method being explored to overcome this is incorporating genes for interleukin-2 (IL-2) or IL-15 within the CAR construct itself, so that there is constant cytokine support to the CAR-transduced cells. This was recently demonstrated in a mouse model of Raji lymphoma at MD Anderson [19]. Further ahead are Kuur Therapeutics and Baylor College of Medicine with a CAR-(natural killer) NK T cell which is engineered to secrete IL-15 [2]; this improves activation under hypoxic conditions and enhances the persistence and anti-tumor activity of the therapy.

Gamma delta-T cell therapy

While gamma delta ($\gamma\delta$)-T cells have proven to be safely activated in patients, they still offer only an average response ratio of 21% and an average clinical benefit rate of only 57%. It is thought that activation-induced $\gamma\delta$ -T cell anergy and reduction in the number of peripheral blood $\gamma\delta$ -T cells post-stimulation with cytokines is likely to be the reason for their poor clinical efficacy [20]. Companies in this space are taking interesting approaches to improve the clinical efficacy of $\gamma\delta$ -T cell therapy against cancer. Gadeta is developing alpha beta ($\alpha\beta$)-T cells engineered to express a defined $\gamma\delta$ TCR (TEG). This essentially combines the efficacy of both types of T cells and increases $\gamma\delta$ -T cell cytotoxicity [21]. Adicet Bio is engineering $\gamma\delta$ -T cells with CARs and TCRs directed to either tumor-specific cell surface targets or intracellular targets [22]. GammaDelta Therapeutics is using the V γ 9V δ 1 cell subset which is mostly found in the thymus and peripheral tissues. While these have more recently been deprioritized in favor of blood derived cells, the company has a proprietary method for selectively

isolating and expanding tissue derived cells to large numbers for clinical use [23].

Tumor infiltrating lymphocyte therapy

Tumor infiltrating lymphocyte (TIL) therapies are heterogeneous; they differ in their CD8+ versus CD4+ T cell ratios, as well as their tumor reactivity and antigen specificity. It is therefore important to pre-select for a tumor reactive population beforehand, and a selection marker is one way of doing this. PD-1, CD137 and CD8 are all potential selection markers that could identify tumor-reactive TILs in a quick and efficient manner [24]. For example, loavance Biotherapeutics is using pre-sorted TILs which can be selected for more specific TILs such as those that express PD-1 and 4-1BB [25].

DC vaccines

In early clinical trials, DC vaccines have been shown to be safe and have the ability to induce CD8+ and CD4+ specific T cell responses, highlighting their considerable potential [26]. But generally, they have shown limited clinical benefit and it seems this may be due to several factors: a reduction in TAA expression by tumor cells leading to immune evasion of the cells; overexpression of immune suppressive barriers such as checkpoint signaling (CTLA-4, PD-1/PD-L1); and defects in the number and functions of DC subsets [27]. To somewhat overcome these issues, some companies are investigating combination of DC vaccines with other immunomodulatory drugs that promote DC activation and T cell function [27], [28]. Others are developing personalized DC vaccines that target a patient's tumor neoantigens [29]. It is thought that using multiple antigens as vaccine targets may overcome tumor escape via antigen-loss [27].

TCR therapy

Safety is still a major issue in this field, and different companies are employing unique methods to solve this. For example, Adaptimmune is developing a TCR against alpha(α)-fetoprotein (AFP) which essentially allows transduced T cells to differentiate

between antigen levels on nonmalignant and cancer cells in patients with hepatocellular carcinoma (HCC) [30].

TCR2 Therapeutics is taking a unique approach to developing TCRs with its T cell receptor fusion construct (TRuC) platform, which allows for recruitment of TCRs to surface antigens without any human leukocyte antigen (HLA) matching. In TRuC-T cells, the tumor antigen binder is conjugated to the whole TCR complex so that the complete TCR machinery can drive full T cell function. This is unlike CAR-T cells which only utilize a single TCR subunit, or TCR-T cells that wholly rely on HLA matching and sufficient HLA expression [31].

COMPLEXITIES IN MANUFACTURING

The high price of pioneering cell therapies like Kymriah and Yescarta is a significant barrier to their large-scale uptake [32]. The prices are in part a reflection of the high manufacturing cost of cellular immunotherapies which involves long, complex, inefficient and poorly scalable multi-step processes.

Current manufacturing processes

CAR-T cell therapy

The first step in manufacturing an autologous CAR-T cell therapy is obtaining a patient's own T cells via leukapheresis; for allogeneic therapy manufacture, T cells are collected from a healthy donor or derived from stem cells instead. T cell separation from peripheral blood mononuclear cells (PBMCs) requires instruments such as CliniMACS Plus and Prodigy systems. Both can be used for enrichment of specific subsets of T cells e.g. CD8+, Tmsc or even naïve T cells [33] [34] [1].

The next step is genetic modification of the T cells to express a CAR specific to a tumor antigen. This can be done by traditional viral-based gene transfer methods using lentiviruses or retroviruses, or through non-viral methods involving DNA-based transposons [35]. For allogeneic CAR-T cell manufacture, gene editing technologies like CRISPR/Cas9 or transcription activator-like

effector nucleases (TALENs) are particularly useful as they can also edit T cells to drop their $\alpha\beta$ TCR, thus reducing the risk of GvHD [36] [37].

After genetic modification, CAR-T cells are expanded to a therapeutic dose. This can be achieved in standard bag-based systems and there are some partially automated platforms available for this, including the Wave 25 bioreactor system [38]; G-rex which is essentially a flask with a gas-permeable membrane base [33]; the Miltenyi CliniMACS Prodigy system or the Lonza Cocoon incubator [39]. However, for cells that have been modified using a transposon/transposase system, expansion is a little more complex, requiring recursive stimulation with irradiated artificial APCs in the presence of IL-2 and IL-21 [33].

Finally, the finished product is infused into the patient.

DC vaccines

Either circulating DCs or monocytes (precursors of DCs that must differentiate into DCs *ex-vivo* before being used to develop a vaccine) are isolated from PBMCs obtained by apheresis. The cells must then undergo maturation which enhances expression of MHC I and II, co-stimulatory molecules and cytokine production. They are then loaded and pulsed with specific TAAs and the resulting vaccine is administered to the patient [40].

Other cellular immunotherapies

The general manufacturing process is much the same for all other cellular immunotherapies [41]–[44], with some subtle differences between technology type. In TCR therapy manufacture, T cells are isolated from PBMCs but need to be engineered to carry TCR α and β chains that recognize intracellular antigen fragments presented by MHC molecules [45]. In TIL therapy manufacture, instead of collecting immune cells via leukapheresis, T cells are extracted from the tumor material itself [24]. Each of

these additional steps increases the complexity of the manufacturing process.

Manufacturing challenges increase the cost of development

Novartis is publicly known to have struggled with commercial manufacture of Kymriah, citing 'product variability' as the main cause [46]. Studies of CD19-targeting CAR-T cell therapies have shown that 5-10% of manufacturing runs are unsuccessful, usually due to inadequate T cell expansion or too few T cells collected by leukapheresis in the first place [47]. Since manufacture of most cellular immunotherapies requires apheresis and *ex-vivo* cell expansion, similar problems are experienced across the board. The process is also very labor intensive and relies on highly experienced personnel. It is therefore no surprise that labor accounts for approximately two thirds of the total cost of goods (CoGs) [48]. Some researchers have developed innovative solutions such as the IL-4 based chimeric cytokine receptor system invented by John Maher's group at Kings College London. The technology enables use of a simple blood draw instead of leukapheresis, however the system has not yet been widely adopted [49].

Supply chain management is infamously burdensome in this field. Each autologous cellular immunotherapy batch is destined for one patient only, so scale up of production is impossible, leaving manufacturers with a very limited economy of scale. Centralized production facilities often used for specialized manufacture have considerable logistical challenges. One might argue that more local production facilities situated closer to individual treatment centers might be a sensible alternative, however, it is simply not feasible in many cases given the investment usually required in setting up and maintaining highly specialized manufacturing facilities and the cost of revalidating all peripheral sites when there is a process improvement or change [48].

Viral vector transduction is also currently costly. There are two main types of viral vectors used: γ retroviral vectors and

lentiviral vectors. The former was the very first type used for CAR-T cell therapy production as it offers a high transduction efficiency and can be easily scaled up [33]. Lentiviral vectors were then developed with some popularity due to their ability to successfully transduce dividing and non-dividing cells with a lower genotoxic profile [50]. However, lentiviral vectors are comparably more difficult and costly to scale up [51].

Technology specific manufacturing challenges

CAR-NK therapy

CAR-NK cells do not expand *in-vivo* therefore, repeated manufacture of therapeutic doses is required for each patient for sustained control of their cancer. Cryopreservation of cell doses, that could be thawed when required for transfusion, would obviate the need for repeated manufacture of new doses [52] but unfortunately, NK cells are too sensitive to the process of freezing and thawing, leading to inferior cell recovery and loss of potency [53]. Evidently, this substantially increases manufacturing costs.

TIL therapy

Like CAR-T cell therapies, TIL therapies have a long lead time, with the manufacturing process taking up to eight weeks. This is because tumor-resected material has to go through multiple microcultures and an individualized tumor recognition assay [54]. Tumor-reactive TILs in combination with lymphodepletion can produce promising clinical results, but the long lead time is not ideal for patients with fast progressing disease and this is associated with high clinical trial dropout rates. To overcome this, manufacturers use young-TILs which are produced from bulk lymphocytes rather than microcultures and also do not undergo tumor recognition screening – this process can significantly speed up TIL therapy lead time [55]. Nevertheless, better streamlined methods of TIL manufacture are still needed. Research efforts for this are ongoing, for example, the Moffitt Cancer Center is trying

to optimize TIL preparation time through 4-1BB agonism [56].

DC vaccines

Neoantigen-targeted autologous DC vaccines are tailor-made for each patient, which presents a particularly challenging manufacturing problem. Each patient's tumor and non-tumor cells must undergo exome sequencing to identify neoantigens; this significantly increases manufacturing costs. [26]. This is not the case with the abovementioned PDC*Line Pharma allogeneic DC vaccine for non-squamous cell lung cancer (NSCLC). The drug product is an off-the-shelf vaccine based on a cell line of plasmacytoid dendritic cells pulsed with peptides derived from target tumor antigens expressed by specific cancers [57].

Improving manufacturing

Automation of manufacturing processes

While automation of cellular immunotherapy manufacturing still demands significant consumable resource [63], [64] and does not reduce production time [35], it does reduce operator variability and allow scale-up. As such, it is estimated that automation could more than halve the CoGs of CAR-T cell therapies. The two automation solutions that currently exist, the Miltenyi CliniMACS Prodigy system and the Lonza Cocoon incubator, allow automated T cell separation, isolation, viral transduction and cell expansion. But other companies are developing scalable systems too; earlier this year Ori Biotech raised \$9.4M for its scalable cell and gene therapy closed manufacturing system platform [58].

Allogeneic cellular immunotherapies

Many of the issues surrounding cellular immunotherapy manufacturing are associated with autologous therapies, and so it is thought that the growth of off-the-shelf allogeneic therapies will eliminate some of the difficulties with scale, cost and lead time.

CONCLUDING REMARKS

The field of cellular immunotherapy is clearly moving at a pace and we will continue to monitor it with great interest. We expect advances will be made in all of the areas mentioned above, and in fundamental immunology including the discovery of novel immune cell types with therapeutic potential. Over the coming decade, we expect more potent and more cost-effective cell therapy solutions to begin penetrating the mainstream of medical treatment across a range of therapeutic areas.

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