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Emerging frontiers in immuno- and gene therapy for cancer

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ABSTRACT

Background aims: The field of cell and gene therapy in oncology has moved rapidly since 2017 when the first cell and gene therapies, Kymriah followed by Yescarta, were approved by the Food and Drug Administration in the United States, followed by multiple other countries. Since those approvals, several new products have gone on to receive approval for additional indications. Meanwhile, efforts have been made to target different cancers, improve the logistics of delivery and reduce the cost associated with novel cell and gene therapies. Here, we highlight various cell and gene therapy-related technologies and advances that provide insight into how these new technologies will speed the translation of these therapies into the clinic.
Conclusions: In this review, we provide a broad overview of the current state of cell and gene therapy-based approaches for cancer treatment – discussing various effector cell types and their sources, recent advances in both CAR and non-CAR genetic modifications, and highlighting a few promising approaches for increasing *in vivo* efficacy and persistence of therapeutic drug products.
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Introduction

With the early successes of *ex vivo*–expanded, autologous tumor-infiltrating lymphocytes (TILs) in patients with metastatic melanoma demonstrating tumor-specific cellular immunity, the field of cellular therapy for cancer treatment, as we largely know it today, was born [1]. Since then, the field has expanded at an astonishing pace. According to the most recent reports on the global development of cancer immunotherapies, there are more than 1300 active cell therapy trials, with chimeric antigen receptor (CAR) T cells comprising approximately one-half of those trials [2].

There have been significant advances in the field over the past 5 years, with regulatory approval worldwide of four CD19 CAR T cells across multiple indications [3–7] and the extension of CAR T targeting B-cell maturation antigen (BCMA) for multiple myeloma [8,9] as well. Despite significant clinical success for some hematologic cancers, especially in the setting of B-cell malignancies, substantial challenges remain. Many patients relapse or fail to respond to CAR T-cell therapy altogether [10]. Beyond hematologic malignancies, CAR T cells face numerous hurdles [11] and still appear to be far from achieving the life-changing effects seen with CD19 and BCMA CAR T cells. Nonetheless, the field is flush with other advancements to address challenges to successful immunotherapy [12]. TILs remain on the precipice of approval for metastatic melanoma [13,14]. While gene engineered T-cell receptors (TCRs) have been met with some safety concerns [15], impressive results in acute myelogenous leukemia (AML) [16] and other cancers [15,17] are starting to be seen. $\gamma\delta$ T-cell therapies have stealthily emerged with promising clinical data [18]. And, returning to the CAR T-cell realm, to mitigate some of the obstacles associated with CART, such as cost [19] and time to infusion [20,21], some centers have begun working on a decentralized, point-of-care (POC) model whereby CAR T are manufactured on site and then infused to the patient [22,23]. This has been made possible by the emergence of new devices, bioreactors and industry sponsors willing to pursue the decentralized POC model [22,23].

As the field of adoptive cellular therapy has expanded, researchers have looked beyond T cells for cellular agents to combat cancer. Natural killer (NK) cells have emerged as a therapeutic option for hematologic malignancies [24], with the potential to be modified with a CAR as well to enable antigen specificity [25]. Furthermore, induced pluripotent stem cells (iPSCs) have been employed to generate

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allogeneic cellular therapies via both NK cells and T cells [26,27]. Hematopoietic stem cells have additionally been proposed as an avenue to overcome resistance to immune checkpoint blockade in solid tumors [28] and similarly could be induced to generate invariant NK T cells, which are ordinarily in low supply and difficult to collect [29]. Finally, researchers have recently turned to myeloid cells in the form of CAR-macrophages [30] and genetically engineered myeloid cells [31] as a novel avenue to combat cancer, particularly for solid tumors.

Focusing on those products or models with the most established clinical experience, in this review we identify four frontiers of cell and gene therapy that are emerging as platforms for the treatment of cancer: (i) CAR T cells; (ii) engineered TCRs; (iii) NK cells and (iv) POC delivery

Each of these four frontiers will be discussed in detail following a broad overview of the current state of cell and gene therapy-based approaches for cancer treatment, including various effector cell types and their sources, recent advances in both CAR and non-CAR genetic modifications and highlighting a few promising approaches for increasing *in vivo* efficacy and persistence of therapeutic drug products. Looking back at lessons learned from some of the earliest clinical trials, we identify key findings that have helped drive the field forward and importantly identify some of the challenges that continue to limit clinical success today. Finally, we provide an overall assessment of the current readiness state of the field from a technological, institutional and regulatory standpoint for its ability to deliver safe, reliable, POC manufacturing of various cell therapy products. We expect that each of these frontiers will play a pivotal role in finding new and innovative treatments for cancer in the coming years.

CAR T cells

As highlighted, CAR T cells stand at the forefront of cellular therapy for cancer. Lessons learned from the earliest experiences with CAR T cells provide a unique foundation to discuss challenges that have arisen and proposed solutions within the CAR T cell field.

CAR T cells are a genetically modified adoptive cell therapy that has revolutionized cancer immunotherapy, particularly for B-cell hematologic malignancies. Originally conceptualized and coined as T-bodies [32,33], current CAR T cells are generally composed of an extracellular antibody binding domain (single-chain variable fragment) coupled with intracellular T-cell–associated transmembrane, costimulatory and intracellular signaling domains. The direct use of an antibody binding domain enables recognition of target antigens that are expressed on the cell surface in a major histocompatibility complex (MHC)-independent manner, obviating the need for antigens to be presented by antigen-presenting cells. Importantly, this facilitates the use of CAR T-cell therapy broadly across diseases with the expressed antigen and patient populations who could potentially benefit from this therapy without restriction to only those with human leukocyte antigen subtypes, the latter a key limitation of TCR-based strategies. These genetically engineered T cells couple antibody-based targeting specificity with T-cell–based cytotoxicity, overcoming endogenous inhibition of the immune system and/or chemotherapeutic resistance. CAR T cells targeting CD19 in B-acute lymphoblastic leukemia (B-ALL) have revolutionized the field by eradicating disease in multiply relapsed and chemotherapy-refractory patients, leading to the Food and Drug Administration (FDA)'s first approval of gene therapy. The field is rapidly expanding—with current efforts focused on addressing limitations of current CAR T-cell constructs, expanding beyond single antigen-targeting, extending the therapeutic index by developing novel CAR T cells against a host of solid and central nervous system (CNS) tumor antigens, along with optimizing engineering and manufacturing strategies for CAR T-cell constructs.

CAR T cells in hematologic malignancies

There are currently four unique CD19 CAR T-cell constructs that are approved by the FDA in adults for many CD19-positive non-Hodgkin lymphomas: Breyanzi (lisocabtagene maraleucel) [7], Tecartus (brexucabtagene autoleucel) [34], Kymriah (tisagenlecleucel) [35] and Yescarta (axicabtagene ciloleucel) [36]. Tisagenlecleucel is the sole construct approved for pediatric B-ALL [3,20], and brexucabtagene autoleucel was recently approved for adults with ALL [37]. More recently, Abecma (idecabtagene vicleucel) [8,38] and Carvykti (ciltacabtagene autoleucel) [9] became the first CAR T-cell products targeting BCMA for patients with multiple myeloma to receive FDA approval.

Lessons learned from the earliest experiences with CD19 CAR T-cell targeting have shed light on important aspects of the toxicity profile, and, more importantly, how to safely manage the complications of cytokine release syndrome and immune effector cell–associated neurotoxicity syndrome. This paved a path for management of novel CAR T-cell constructs and associated toxicities—which can be life-threatening [39]. Study of longer-term toxicities, including the impact of long-term B-cell aplasia, is under active investigation [40].

A particularly notable outcome from CD19 CAR T cells was in their ability to not only induce remission but potentially also lead to long-term durable remission [7,20,35]. The loss or downregulation of CD19, or an alternate B-cell antigen (e.g., CD22), subsequently emerged as one of the primary causes of disease relapse following single-antigen targeting strategies [41–44]. Thus, future directions in B-cell targeting focus on combinatorial treatment strategies.

Among the host of mechanisms of resistance to CAR T-cell therapy [10] was also the recognition that the mere ability to manufacture CAR T cells was highly dependent on patient-specific factors, inclusive of the impact of prior therapy, absolute lymphocyte counts and T-cell functionality [45]. Indeed, CAR T-cell fitness plays a role in CAR-T expansion and persistence, characteristics that are needed for both optimal antitumor killing and long-term surveillance—factors that will continue to play a role as novel CAR T-cell constructs are tested.

Beyond the successes in ALL and non-Hodgkin lymphoma, efforts in CAR T cells targeting Hodgkin lymphoma targeting CD30 have shown promising results in early-phase clinical trials of heavily pretreated patients [46]. In contrast, efforts in targeting T-cell lymphoblastic leukemia (T-ALL) and AML have been met with significant early challenges. T-ALL presents the unique paradox of selecting an appropriate target that will not result in CAR T-cell fratricide, as well as issues related to contamination of the CAR T-cell product upon collection and the toxicity related to long-term T-cell aplasia [47]. For AML, the co-expression of a target myeloid antigens on both leukemic cells as well as hematopoietic stem cells (e.g., CD33 or CD123) raises the critical concern for myeloid aplasia as a potential toxicity of AML-directed CAR T cells, necessitating availability of a stem cell source for salvage of aplasia [48]. Early experiences suggest also that the tumor microenvironment for CAR T cells in AML, the patient population, prior therapy and fitness of generated CAR T cells may differ vastly from the experiences in ALL and will require ongoing optimization to have an active AML CAR T-cell construct [49].

Early CAR T-cell experiences in solid tumors

Expanding upon the tremendous success of CAR T cells in hematologic malignancies, a host of strategies to explore the therapeutic potential of CAR T cells in solid tumors is under investigation. This section will focus on the current state of the art of CAR T cells in solid tumor with regards to antigen selection, CAR T-cell efficacy and next-generation strategies.

Target antigen

Finding an optimal target for CAR T-cell targeting in solid tumors remains a key challenge to optimizing this adoptive cell-based

strategy. Unlike B-cell targeting, which has the benefit of targeting an antigen where loss of normal cells can be easily supported, targeting in solid tumors is more nuanced. On the one hand, relevant targets are expressed on several different histologies, offering the potential for a single novel construct to have activity against a wide range of tumors and benefit a larger population. However, it is simultaneously difficult to select a target that is cancer-specific without co-expression on normal tissues and does not provoke significant on-target/off-tumor toxicity. This tension is clearly illustrated by the experience of using CAR T cells to target HER-2 (ERBB2), where differing trials have variably reported either overwhelming and occasionally fatal toxicity [50] or lack of clinical efficacy [51], potentially due to differing affinity of the CAR T cells for their targets. While a full rendering of recent CAR T-cell clinical trials for solid tumors and their results is outside the scope of this paper, we do highlight a few targets with encouraging early results which continue to be actively investigated.

The disialoganglioside GD2 has proven to be an effective immunotherapeutic target in neuroblastoma, as evidenced by efficacy of the anti-GD2 antibody dinutuximab in the landmark Children's Oncology Group trial ANBL1221. CAR T cells targeting GD2 have resulted in complete remissions in a subset of patients with neuroblastoma [52,53]. A more recent a trial combining an anti-GD2 CAR T cells with programmed death-1 (PD-1) inhibition failed to show a synergistic effect but did show that GD2-directed CAR T cells induced sustained disease control in a number of patients [54]. GD2 expression also has been reported in melanoma [55], Ewing sarcoma [56], and a subset of osteosarcomas [57], soft-tissue sarcomas [58], and other solid tumors [59]. Based on these preclinical observations and early clinical trial observations, clinical trials of GD2-directed CAR T cells are either actively recruiting or in development targeting glioma, osteosarcoma, neuroblastoma and other solid tumors that screen positive for GD2 expression.

Mesothelin is a differentiation antigen that is expressed on a wide range of solid tumors [60] with relatively minimal expression on normal tissue. Based on this promise, a number of clinical trials across a range of histologies including mesothelioma, ovarian cancer, pancreatic cancer and other relapsed mesothelin-positive solid tumors have opened in recent years. While results are only beginning to be reported, several cases demonstrating safety have begun to be reported [61], and one such trial of mesothelin-targeted CAR T cells delivered intrapleurally demonstrated safety and preliminary efficacy in a phase 1 trial in combination with immune checkpoint inhibition [62]. For a more comprehensive review of CAR T cells in solid tumors, we would refer to the excellent recent review by Bagley and O'Rourke [63].

CNS tumors present the added challenge of designing a CAR T-cell construct that can traffic through the blood–brain barrier. CAR T cells targeting either GD2 or B7-H3 have been explored in CNS tumors. Novel constructs show promise, but the toxicity profile within the CNS will need to be considered very carefully [64–66]. Indeed, even models targeting GD2 in non-CNS tumors have reported potentially fatal neurotoxicity [67]. Furthermore, consideration should be given to the method of administration, as, for example, Donovan *et al.* [68] have proposed that intrathecal administration of CAR T cells targeting EPHA2, HER2 and IL13Rα2 may be efficacious for circumventing the blood–brain barrier and directing therapy to the tumor site, potentially also preventing off-tumor effects that may occur with intravenous administration.

CAR T-cell expansion and trafficking in solid tumors

Indeed, trafficking of CAR T cells in solid tumors may be challenging for a host of reasons, including repellant chemokine gradients [69] and physical barriers. Unique approaches to administration of CAR T cells to direct to sites of disease are being explored, as previously mentioned (e.g., intrapleural [68] and intra-ventricular [66]). However, even if CAR T cells are able to traffic to the site of disease and invade a solid tumor, the tumor microenvironment may promote localized CAR T-cell exhaustion [70] due to the presence of tumor-associated macrophages and myeloid-derived suppressor cells [71], expression of inhibitory ligands such as PD-L1 by tumor and stromal cells and tissue hypoxia which is unfavorable for T-cell metabolism.

In broad terms, two parallel strategies have been proposed to overcome these unique challenges posed by solid tumors. Next-generation CARs in solid tumors with intrinsically altered stimulatory or inhibitory signaling structures, or which are engineered to autonomously secrete cytokines to aid in trafficking or activation, have been proposed in a wide variety of forms [72,73]. Alternately, co-administration of CAR T cells with agents that condition the tumor microenvironment to enhance T-cell fitness (such as via immune checkpoint inhibition) [74] and eliminate immunosuppressive features (such as targeting tumor metabolomics to reprogram immunosuppressive myeloid cells) [75] are also avenues being actively explored.

Challenges and considerations for future iterations

While CAR T cells have been highly effective for B-cell malignancies, challenges still remain, as many patients fail to respond or eventually relapse, and many diseases continue to not respond to CAR T-cell therapy (Table 1) [50,53,60,61,71,73,75–93]. Proposed solutions largely involve either further modification of the CAR T cell itself to

Table 1
Challenges and proposed solutions of CAR T cells.

Challenges in CAR T-cell strategies	Proposed solution	References
Optimal target antigen	• Identifying target antigens which are relevant across multiple histologies (i.e., GD2, mesothelin, HER2)	[50,53,60]
Antigen downregulation	• Co-administration of multiple CAR T cells • Dual-targeting CAR T cell	[76–83]
Failed expansion/persistence	• Altered co-stimulatory motifs/signaling machinery • Selection based on CD4/CD8 ratio or specific central-memory phenotype. • Overcoming T-cell exhaustion via combination with immune checkpoint inhibition	[73,84-87]
CAR T-cell reactivity	• Further study is necessary to optimize second and subsequent CAR T-cell infusions • Role in increased lymphodepletion for patients previously treated with CAR T cells	[88–90]
Lack of CAR T-cell trafficking to tumor	• Locoregional CAR T-cell infusion • Forced CAR T-cell expression of chemokine receptors	[61]
Immunosuppressive tumor microenvironment	• Coadministration of immune checkpoint inhibitors • Modulation of CAR T-cell to remove PD-1–based inhibition • Modification of CAR T-cell metabolism • Expression of cytokines to promote effective T-cell activity • Combination with agents to deplete or reprogram immunosuppressive myeloid-derived suppressor cells • Reprogramming tumor metabolomics	[71,75,87,91-93]

CAR, chimeric antigen receptor.

overcome these challenges, or combination with other agents to modulate the tumor microenvironment.

The most common cause of treatment failure is immune escape via downregulation of the target antigen, as seen in experiences with CD19- or CD22-directed CAR T cells [10,94]. Dual-targeting CAR T cells, such as bispecific anti-CD19/CD22 or anti-CD19/CD20 CARs, have been proposed as one way to overcome this immune escape mechanism and are being explored in a number of early phase clinical trials (NCT03241940, NCT04007029, NCT03233854, NCT04186520, NCT03448393, NCT03330691, NCT04049383, NCT05098613). A critical component to dual-targeting CAR T cells, however, will be to confirm that dual efficacy can be established. Strategies such as co-infusion, co-transduction or incorporation of two single-chain variable fragments onto a single vector are being explored and, as experienced is gained, it will be important to look at how these novel strategies perform in key determinants of CAR T-cell outcomes (e.g., persistence, duration of remission, toxicity). Several early trials using dual-antigen bispecific targeting of CD19/CD22 [79,80] or CD19/CD20 [81] are beginning to be reported, with encouraging results in terms of overall remission rates and toxicity profiles. Limitations in regard to the efficacy of dual targeting and/or CAR T cell persistence using these constructs reveal opportunities for ongoing optimization of these novel strategies [79,80].

A smaller but still substantial proportion of patients will fail to have initial engraftment or expansion/persistence, potentially due to rapid accumulation of markers of T-cell exhaustion [88]. This is particularly true for solid tumors, where CAR T cells must overcome a hostile tumor microenvironment, and similarly the bone marrow microenvironment of hematologic malignancies, where CAR T cells have not yet been effective needs to be further explored [95]. Further engineering of the CAR T cell to overcome these factors by constitutive expression of activating cytokines (NCT03635632, NCT04377932, NCT03721068), or combination with immune checkpoint inhibitors (NCT04134325, NCT04205409) also have entered early-phase clinical trials. As trafficking to the site of solid tumors is a substantial barrier, and particularly in CNS tumors, locoregional administration of CAR T cells has been proposed as one way to overcome this challenge (NCT04185038, NCT03638167, NCT03500991, NCT03696030), as has co-expression of cytokine receptors such as CCR4 (NCT03602157). Furthermore, the impact of the generation of anti-CAR antibodies and the immunogenicity of CAR T cells is only beginning to be evaluated, and further exploration of this question will be necessary to better understand how to propose second and subsequent CAR T cell infusions [88–90].

The impact of manufacturing on CAR T functionality and patient-specific outcomes is also under active investigation. More laborious manual bag/culture methodologies are slowly being replaced by automated closed-system devices, as discussed to follow. The centralized model of manufacturing, under which many of the first CD19-directed CAR T cells were approved, is also being replaced as decentralized models become more readily available. Most importantly, however, is the recognition that the ultimate CAR T-cell infusion product is reflective of the process—and even seemingly-minor changes in manufacturing may impact the toxicity profile. The most extreme example of this emerges from the JCAR015 ROCKET study, where increased rates of cerebral edema were seen in patients receiving CD19/28z CAR following minor changes in manufacturing from the predecessor trial with this construct [96]. Another study with CD22 CAR T cells has demonstrated that even minor changes in selection of the starting apheresis material can change the toxicity profile [97]. Additionally, several early-phase clinical trials are selecting specific T-cell attributes, such as a memory phenotype, to explore whether this will result in increased activity (NCT03389230). Similarly, for patients who have previously received an allogeneic bone marrow transplant, the question of whether generating CAR T cells from their allogeneic donor is an open question under investigation (NCT01087294, NCT04556266). Investigations into attributes of the individual CAR T cell products will shed light on individual elements that impact both efficacy and toxicity and/or persistence [98–100].

Finally, toxicity from CAR T cells, including cytokine release syndrome, remains a significant source of morbidity and even mortality, and strategies to mitigate these toxicities are also under close investigation [101] (NCT04148430). Novel constructs with so-called “on” and “off” switches [102] and/or incorporation of targetable receptors (e.g., epidermal growth factor receptor) or use of pharmacologic approaches (e.g., dasatinib) [103], to facilitate more precise control may help facilitate fine-tuning of toxicity and response. As these therapies evolve, a greater emphasis on improving the safety profile while maintaining efficacy will be a key goal. The new frontiers for CAR T are expanded access to patients, continued expansion beyond CD19 CAR T cells, off-the-shelf CAR T and improvements in CAR T targeting solid tumors.

Engineered TCRs

The approval of the first CAR T-cell therapy in 2017 codified adoptive T-cell therapy as a viable modality for treating cancer patients. Due to the challenges associated with CAR T-cell therapy, alternative methods to harnessing T-cell–based cellular therapy against cancer are being reconsidered. TILs and engineered TCRs are other clinical-stage adoptive T-cell therapies that have yet to be (but may soon be) approved [104]. Generation of TILs relies upon the *ex vivo* expansion of the patient’s tumor-specific endogenous T-cell repertoire. The challenges in identifying and expanding endogenous tumor reactive T-cell clones has limited the utility of TIL therapy, although TILs have still demonstrated significant promise as reviewed elsewhere [105]. Here we will more closely consider engineered TCR strategies currently under development and the unique advantages and disadvantages which may be afforded by this treatment modality.

Engineered TCRs are generated via *ex vivo* transduction of tumor-selective antigen-specific TCR alpha and beta chains into the patient or donor T cells. Functioning as a canonical TCR, engineered TCRs can target extracellular antigens as well as intracellular antigens presented on the cell surface by MHC, a target that is typically inaccessible to traditional CARs. Despite this apparent advantage, engineered TCRs face several unique challenges that have slowed progress towards an approved therapy.

Most TCR-based approaches use sequences identified in either healthy donors or patients with cancer that target self-antigens selectively expressed on tumor cells. These TCRs often lack the affinity required for robust T-cell activation because thymic selection eliminates T-cell clones with high-affinity TCRs against self. TCR affinity can be enhanced by screening a library of TCR variants for high-affinity binders; however, these variants also can possess novel and sometimes lethal “off-target” specificities [15]. Another potential source of “off-target” TCR activity can arise from the mispairing of the exogenously expressed TCR alpha or beta chain with its endogenously expressed counterpart. Protein-engineering strategies have been developed to favor formation of the desired exogenous TCR; however, none have been able to eliminate mispairing [106].

A preclinical publication in 2012 demonstrated that gene editing offers an elegant solution [107]. Provasi *et al.* [107] used zinc finger nucleases to knock out the endogenous TCR alpha and beta genes in primary human T cells and lentiviral gene transfer to stably express a TCR specific for Wilms tumor 1 antigen (WT1), a tumor antigen highly expressed on a number of cancers. They compared the activity of the edited cells with non-edited WT1 TCR T cells generated by the same lentiviral gene transfer process. The edited cells killed WT1-positive target cells more potently than their unedited counterparts and demonstrated superior *in vivo* anti-tumor activity. The authors also showed that eliminating the endogenous TCR eliminated allogeneic activity in a mixed lymphocyte reaction and graft-versus-host

disease model. The latter result is important because it shows the potential for safely administering TCR alpha/beta edited allogeneic T cells to patients. This seminal work has been reproduced using other gene editing platforms such as CRISPR/Cas9 and show that a TCR-alpha/beta double knock-out increases the potency of engineered TCR T cells and eliminates the risk of graft-versus-host disease [108].

Until recently, the clinical evaluation of engineered TCRs was limited to non-edited T cells and has been largely disappointing, with a few noteworthy exceptions. Patients with AML who relapse after allogeneic stem cell transplant have a poor 2-year survival rate. WT1-targeting TCR T cells generated from the stem cell donor's Epstein Barr virus–specific CD8+ T cells were given prophylactically to high-risk patients with AML with no signs of disease 28 days after stem cell transplant [16]. Epstein Barr virus–specific CD8+ T cells were selected to minimize the risk of graft-versus-host disease and exploit the enhanced persistence of viral specific memory T cells. All 12 patients who received TCR T cells were alive, required no additional AML treatments and had no evaluable disease at a median time of 44 months between the first infusion and follow-up. In contrast, the mortality and relapse rates of high-risk patients with AML that were also disease free 28 days post-transplant was 39% and 28%, respectively, at a similar follow-up time.

NY-ESO-1 targeting TCRs are, to our knowledge, the only affinity enhanced TCRs with an acceptable safety profile. Other affinity enhanced TCRs have resulted in either severe "on-target" or "off-target" toxicities [15]. NY-ESO-1 targeting TCR T cells also have demonstrated clinical responses in patients with synovial sarcoma and multiple myeloma across several trials [15]. Recently, Tmunity evaluated the safety and efficacy of autologous CRISPR/Cas9-edited TCR-T cells in two patients with myeloma and one patient with sarcoma [17]. Knock-out targets included TCR-alpha, TCR-beta and the inhibitory T-cell receptor PD-1, and lentiviral gene transfer was used to stably express the NY-ESO-1 targeting TCR following editing. Editing frequency in the final product was 45% for TCR-alpha, 15% for TCR-beta and 20% for PD1, and transduction efficiency was less than 5%. In patients, the edited cells could be detected for up to 9 months after administration, and most adverse events could be attributed to the lymphodepleting chemotherapy that was given before TCR-T-cell treatment. The best clinical response was stable disease in two of the three patients. This study garnered attention because it showed that CRISPR/Cas9 edited T cells could persist in patients with cancer despite theoretical concerns that lingering Cas9 protein in the edited cells is immunogenic. The true potential of this therapy will be realized when patients are treated with TCR T cells that exploit advances in multiplex editing efficiency and TCR expression. Despite the demonstrated promise of exogenous TCRs, beyond the aforementioned potential toxicity, the biggest drawback of this approach is the limited breath of targeted alleles, which ultimately limits the various treatments—at least the initial ones—to people of select races and ethnicities.

In summary, potential therapies with engineered TCRs are an emerging frontier of immunotherapy for cancer. CRISPR/Cas9 gene editing has the potential to push TCR-T cell therapies toward an approval by enabling multiplex gene editing at high efficiency. Knocking out both TCR-alpha and TCR-beta eliminates off-target activity from TCR mispairing and enables the creation of an allogeneic T-cell product from healthy donors. Allogeneic TCR-T cells have the advantage of immediate, off-the-shelf availability and are not limited by intrinsic T-cell dysfunction associated with certain cancers. Finally, multiplex editing enables the inclusion of additional edits that have the potential boost expansion, persistence and function of TCR-T cells in the tumor microenvironment [109].

Natural killer (NK) cells

While T-cell–based adoptive cellular therapies are furthest along in development, interest within the field is growing to expand the repertoire of cellular therapies beyond T cells. Of these, NK-cell–based therapies have generated some of the greatest excitement and will be reviewed in greater detail.

NK cells have been used clinically for more than 30 years [110]. NK cells are cytotoxic lymphocytes that have important roles in both the innate and adaptive immune responses. NK-cell activation is a result of coordinated regulation of both inhibitory and activating receptors that enable NK cells to recognize and destroy targets via interactions with cells either lacking appropriate MHC class I expression or foreign ("non-self)" MHC class I expression while maintaining self-tolerance mechanisms. Since their antigen recognition is not limited to peptides presented in the context of MHC, they are also well suited for use in an allogeneic fashion. In addition, NK cells recognize stressed cells that arise from infections and tumor formation [111]. NK cells eliminate target cells through the release of cytolytic granules or by antibody-dependent cellular cytotoxicity (ADCC). As such, they are an attractive cellular therapeutic because they can effectively target tumors without causing toxicities like graft-versus-host disease.

In hematopoietic transplantation and hematologic malignancies, the use of NK cells has yielded promising clinical outcomes. In a study with patients with AMLs, five of 19 patients treated with haploidentical NK cells achieved a complete response [24]. Clinical responses were associated with greater circulating NK cells, increased NK cell cytotoxicity and KIR ligand mismatched. However, their success as a cellular immunotherapy has been inconsistent, often marked by lack of persistence/expansion in vivo and decreased function [112]. In addition, NK cells isolated from patients with cancer often are dysfunctional as a result of interactions within the tumor microenvironment [113]. Therefore, much of the current research involving NK cells seeks to overcome these barriers. While a full review of the therapeutic use of NK cells will not be discussed here, there are several excellent recent reviews on this topic [114–116]. In Table 2 [117–124], we highlight new developments in the use of NK cells that provide give a glimpse of where the field of NK-cell therapy might be headed.

Sources of NK cells

There has been considerable debate about the optimal source of starting material for manipulating NK cells. Traditional sources of NK cells include autologous sources from peripheral blood and allogeneic sources from either peripheral blood or umbilical cord blood [125]. While these sources have been used extensively for decades, problems like poor yields from the leukapheresis collection of cells, contaminating cells and significant donor heterogeneity have led to the development of allogeneic, ready-to-use "off-the-shelf" sources to alleviate these problems [27]. For example, the transformed NK cell line NK-92 has been used as an off-the shelf product to overcome the technical difficulties of gene transfer into NK cells [121]. In their animal model of lymphoma, Oelsner *et al.* [121] found that although the CAR costimulatory signaling domains influenced NK-cell cytokine secretion and expression of inhibitory/exhaustion markers, the CAR NK-92 cells demonstrated augmented cytotoxicity and anti-tumor activity. ImmunityBio (formerly Nantkwest, Inc.) is now modifying this cell line with high binding affinity receptors (haNK) CARs (t-haNK), and memory-like cells (M-ceNK) [126]. Cryopreserved haNK have been tested in early-phase QUILT trials targeting a number of different cancer types.

There are other sources that may be able to overcome many of the aforementioned issues. These include NK cells derived from $CD34^+$ hematopoietic stem cells, iPSCs and embryonic stem cells [127]. These sources have been proposed to provide a more homogeneous source and achieve larger, clinically relevant doses. iPSCs generated during the manufacturing process are thought to be quite amenable to genetic manipulation during the differentiation process [128]. Zhu

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Table 2
Antigen recognition by NK cells.

Cell type	Method of antigen recognition	Documented targets	Comments	Reference
NK cell, peripheral or cord blood derived	NKG2D	MICA/B, RAET1, ULBPs, MHC class 1 molecules	Targets expressed on infected, damaged or transformed cells	[117]
	Chimeric antigen receptor	CD19	NK cells also expressed IL-15 and an inducible caspase 9	[118]
	Chimeric antigen receptor	NKG2D	Targets NKG2D on myeloid derived suppressor cells	[119]
NK-92 cell line	Antibody-dependent cell-mediated cytotoxicity	EGFR, HER2/neu	NK cells were engineered to express a high-affinity CD16 allele	[120]
	Chimeric antigen receptor	CD19	Cytotoxic to leukemia and lymphoma targets	[121]
	Chimeric antigen receptor	GD2	UniCAR NK cells designed with an on/off mechanism	[122]
iPSC-derived NK cells	Chimeric antigen receptor	Mesothelin	NK-cell activation domains improved NK-cell cytotoxicity and expansion	[123]
	KIR-negative NK cells	Various cancer cell lines	Manufacturing designed to provide large scale allogeneic use of NK cells	[124]

EGFR, epidermal growth factor receptor; IL, interleukin; iPSC, induced pluripotent stem cell; MHC, major histocompatibility complex; NK, natural killer.

and Kaufman [128] have outlined a protocol for generating NK cells from several iPSC cell lines, including human fibroblasts, peripheral blood mononuclear cells and CD34+ cells from umbilical cord blood. They developed a two-stage, feeder-free, culture system with an expansion phase using interleukin (IL)-21–expressing artificial antigen-presenting cells. The resulting NK cells were CD45+CD56+ and expressed a number of activating receptors, suggesting that they were fully mature NK cells that exhibited similar cytotoxicity to peripheral blood–derived NK cells. This technology is being actively translated into the clinic, as discussed to follow.

Zeng *et al.* [124] have demonstrated that a widely used human embryonic stem cell line, H1, can be used to generate NK cells. To generate the NK cells, H1 embryonic stem cells were first co-cultured with modified OP9 bone marrow stromal cells to differentiate the cells into CD34+ cells. The CD34+ cells were subsequently cultured with OP9 cells expressing the Notch ligand Delta-like-1, stem cell factor, FMS-related tyrosine kinase 3 ligand and IL-7. This culture system generated highly pure and potent NK cells that lacked killer immunoglobulin-like receptors. This approach is of interest because it represents a nearly infinite source of off-the-shelf NK cells that, because they are from a single donor, lack donor-to-donor heterogeneity. These studies, sponsored by both industry and academic cell therapy centers, are in the process of being translated to the clinic.

NK-cell modifications

Gene modification and new approaches of activating NK cells are also providing opportunities to expand the use of NK cells clinically. These new strategies seek to overcome many of the barriers that limit NK cell efficacy, such as increasing NK-cell proliferation and persistence, improving receptor NK-cell signaling and effector function, increasing antigen specificity, optimizing sources of NK cells and overcoming tumor-mediated immunosuppression. Here we outline a few recent strategies in detail.

Genome editing techniques: chimeric antigen receptor NK cells

As seen with the CAR T-cell therapies, there is tremendous interest in generating CAR NK cells. Unlike CAR T cells, CAR NK cells are thought to cause less cytokine release syndrome. [25] The first clinical data from a clinical trial using CAR NK cells were recently published by the Rezvani group in patients with relapsed or refractory CD19-positive cancers [118]. In this groundbreaking trial, NK cells derived from cord blood were transduced with a combination of an anti-CD19 CAR, interleukin-15 (IL-15) to promote expansion and persistence in vivo and inducible caspase 9 as a safety switch. The trial was designed for a single infusion at escalating doses of 1×10^5, 1×10^6, and 1×10^7 cells per kilogram. The majority of patients (8/11) responded to the treatment, including seven complete remissions. The responses appeared to be independent of cell dose, although the number of subjects treated was small. Interestingly, the team found that CAR NK cells demonstrated superior persistence over the traditional use of non-engineered NK cells. CAR NK cells were observed at low levels even at 12 months after infusion, likely a result of the IL-15 expressed by the gene-modified NK cells. Since this groundbreaking study, numerous clinical studies have been initiated testing CAR NK cells for a variety of cancers. These studies, along with a number of emerging pre-clinical studies, have been elegantly outlined in a review by Wang *et al* [25].

Parihar *et al.* [119] designed a unique human CAR NK cell that targeted the immunosuppressive tumor microenvironment rather than a specific tumor antigen. NK cells derived from peripheral blood were cultured with feeder cells and transduced with a CAR construct containing the activating receptor of NKG2D fused to the intracellular ζ chain of the T-cell receptor. These NKG2D.ζ NK cells targeted immunosuppressive myeloid-derived suppressor cells but not normal tissue expressing NKG2D. Using these cells in a murine model, the authors demonstrated that NKG2D.ζ NK cells could eliminate intratumoral myeloid-derived suppressor cells, recruit GD2 CAR T cells to the tumor via cytokine secretion and subsequently improve the anti-tumor activity of the GD2 CAR T cells. Finally, Nkarta Therapeutics has developed CAR constructs with the OX40 costimulatory domain, CD3ζ signaling domain and membrane-bound IL-15 [129]. Two products, NKX101 (NKG2D) and NKX019 (CD19), are moving into early-phase clinical trials [130,131]. A phase 1 clinical trial using NKX101 for patients with relapsed/refractory acute AML or myelodysplastic syndromes currently enrolling patients (NCT04623944).

In addition to donor-derived NK cells, NK cells generated from other sources like the NK-92 cell line and iPSCs also have been engineered to express various CARs. Li *et al.* [123] demonstrated that a CAR with three engineered NK activation domains, (i) a NKG2D transmembrane domain, (ii) a 2B4 co-stimulatory domain and (iii) a CD3ζ signaling domain provided superior anti-tumor activity than NK cells containing the typical T-cell activation domain found in conventional CAR T cells. Furthermore, the NK-specific enhancements led to improved expansion and survival of the transduced iPSC-NK cells in animal models. As such, this approach provides potential for an effective off-the-shelf product with improved persistence and anti-tumor activity. Mitwasi *et al.* [122] have generated another off-the-shelf NK-type product using the NK-92 cell line. The “UniCAR” NK-92 were engineered with a construct consisting of an extracellular single-chain fragment variable antibody directed against a E5B9 peptide epitope, a CD28 transmembrane and costimulatory domain, and a CD3ζ signaling domain. The UniCAR was engineered with an antigen target module (TM) that targeted the antigen disialoganglioside GD2 fused to the E5B9 peptide epitope recognized by the UniCAR. Since the UniCAR-NK cells are only active in the presence of the TM, removal of the TM essentially renders the cells inactive. By fusing the

two antigens, the team improved the specificity of tumor-UniCAR interactions in a controllable fashion.

Genome-editing techniques: non-CAR

The difficulty of optimal gene delivery into NK cells has been one of the many obstacles that have prevented the advancement of NK cell therapy. However, as technologies in gene delivery, like the CRISPR/Cas9 system, have matured, it is expected that this problem can be properly mitigated [132]. A clear example of this is the recent study by Pomeroy *et al.* [133] that used the CRISPR/Cas9 genome-editing technology to knock down inhibitory signaling molecules in NK cells. Two genes, ADAM17 and PDCD1, were delivered by electroporation into activated peripheral blood mononuclear cell–derived NK cells. ADAM17, a protein in the disintegrin and metalloproteinase family, rapidly cleaves the activating receptor for ADCC CD16a. The engagement of PD-1 on the surface of NK cells with its ligand PD-L1, often overexpressed on tumor cells, leads to a reduction in NK-cell cytotoxicity. These modified NK cells demonstrated improved ADCC-mediated target killing as well as superior cytotoxicity, mediated by increased degranulation and cytokine production. Another recent approach has been to gene-modify iPSC-NK cells. An elegant study from Kaufman's group demonstrated that iPSC-NK cells engineered to express a non-cleavable form of CD16a led to increased ADCC-mediated activity compared with unmodified iPSC-NK cells [134]. The team found that the combination of the engineered cells with specific monoclonal antibody in multiple cancer types led to an effective and improved treatment in animal models. This pre-clinical work was done in partnership with Fate Therapeutics. The engineering of iPSC-NK cells strategy has demonstrated significantly clinical utility such that Fate Therapeutics has now developed a pipeline of engineered NK cells including NK100 (donor NK cells cultured with a GSK3α/β inhibitor), FT500 (iPSC derived NK cells), FT516 (iPSC-NK cells expressing a non-cleavable CD16 receptor [hnCD16]), FT596 (iPSC- CAR NK cell expressing CD19 CAR, hnCD16 and an IL-15 receptor) and FT538 (iPSC-NK cell expressing hnCD16 and lack of CD38 expression) [135].

New avenues

Among the many exciting areas of research in NK cell biology, the regulation of immunometabolism of NK cells is an area of emerging investigations that may impact NK cell therapy by providing valuable information on the activation, differentiation and persistence of NK cells. Cytokine-induced glycolysis and oxidative phosphorylation are critical for NK cell activation (reviewed by O'Brien and Finlay [136]). This area is also particularly relevant in that understanding the metabolic pathways that control NK activation will guide new efforts to counter the nutrient-poor tumor microenvironment. For example, Kedia-Mehta *et al.* [137] demonstrated that the interactions of NK cells with tumor cells led to the upregulation of the IL-2 receptor, CD25. The induced expression and signaling through CD25 led to the upregulation of the metabolic regulators mTORC and c-Myc, resulting in prolonged NK-cell survival due to high rates of glycolysis and oxidative phosphorylation. c-Myc appears to be particularly important, as it integrates multiple metabolic pathways to increase survival, proliferation, activation and functional responses [138,139]. Further demonstrating the importance of cellular metabolism, Zhu *et al.* found that metabolic reprogramming of iPSC-derived NK cells via deletion of a negative regulator of IL-15 (CISH) led to increased glycolytic activity and maximal mitochondrial respiration [140,141]. These data suggest that engineering NK cells with optimal metabolic function is a prudent approach for future studies.

Exercise also has the potential to positively regulate immunometabolism of NK cells. Exercise has been shown to increase cytotoxicity and trafficking to tumors [142,143]. In humans, both CD56dim and CD56bright NK cells were rapidly mobilized into the peripheral blood and in some cases increased blood counts as much as 5- to 10-fold [144,145]. Exercise can also counter the effects of obesity, as excess lipid accumulation has been shown to cause NK-cell dysfunction [146,147]. For these reasons, exercise has been proposed as a novel way to improve cancer immunotherapy [148]. Exercise can be used to increase the absolute numbers and potential quality of NK cells from leukapheresis products and/or long-term regular exercise regimens could be incorporated into treatment regimens of patients receiving NK cell products. In summary, the mechanisms revealed from studies on NK cell immunometabolism and new insights on function, activation, and survival can be applied in both *ex vivo* culture systems and *in vivo* manipulations. As such, the further understanding of this area has significant potential to improve responses to NK cell therapies. NK cells remain an emerging frontier for immunotherapies targeting cancer, with CAR NK cells currently demonstrating the most potential as an off-the-shelf treatment for a number of cancers.

POC manufacturing

In addition to the limitations intrinsic to the specific cellular therapies detailed throughout our review, the specialized expertise required to manufacture these novel treatments impose a significant obstacle to delivery outside of tertiary academic medical centers. Addressing this hurdle will be critical to ensuring that novel therapies are accessible so that all patients may benefit.

A decentralized model of manufacturing for cell and cell/gene therapy products (advanced therapy medicinal products, or ATMPs) is one of the most debatable topics in the cell therapy community. There are at least two approaches to decentralized manufacturing in cell therapy, such as regional (one manufacturing plant produces cell therapy products for several hospitals locally in specific region) and hospital-based (product manufactured in the hospital at POC). Even though there is no doubt that the decentralized POC manufacturing model has many potential benefits (reviewed in [149–151]) and could be the most attractive for autologous cell and cell/gene therapies, it is the most challenging model to implement in practice. Today, a clear path for implementing a POC model in the hospital setting does not exist. To help us understand where we are standing today with implementation of ATMP manufacturing at POC and to highlight remained challenges, we propose to assess the implementation through technology readiness level (TRL). The recent developments in ATMPs manufacturing at POC (Table 3) [152–154], give us a hope for successful implementation of this model in the future.

Technological readiness

The launch of the CliniMACS Prodigy system by the German company Miltenyi Biotec in 2016 was revolutionary for the cell and gene therapy field. Combining cell separation/selection and cell culture (expansion) functionalities in one closed-system device brought us much closer to the reality of an ATMP manufactured at POC. The Prodigy system set a new standard for the industry and healthcare, introducing the concept of all-in-one multifunctional automated device to enable Current Good Manufacturing Practice (cGMP)-compliant POC manufacturing "in-the-box." In the last 5 years, the Prodigy has been validated for multiple ATMP manufacturing processes and was used in few dozens of clinical trials, mostly in cellular immunotherapy of cancer. A basic Prodigy configuration has now been enhanced by the addition of an electroporator and adherent cell culture system.

In the last several years, Miltenyi has done significant work to prove that the use of Prodigy for manufacturing ATMPs such as CAR T cells results in a consistent product independent of facility, operator and starting material [22,23]. About 3 years ago, the German authority (Paul-Ehrlich-Institute) granted manufacturing permission for use

Table 3
Examples of recent advances in cell and gene therapy for technology readiness level assessment.

Level of Readiness	Examples
Technological	• Automated closed system multifunctional device, which could allow entire manufacturing process in environment of grade C-D (ISO7-8) – CliniMACS Prodigy (Miltenyi Biotec) • Product testing: POC flow cytometry (Accellix) • Flexible modular prefab facilities
Regulatory	• FDA readiness to issue multiple biologics license application (BLA) for the same product to each physician/hospital separately [152] • Allowance of German authority (PEI) to manufacture CAR T product entirely in class C environment, using closed system automated device [153] • Spanish AEMPS approval of Hospital Exemption for anti-CD19 CAR T therapy by Hospital Clínic Barcelona [154] • FACT/JACIE standards for Immune Effector Cells and Common Cell Therapy Standards
Institutional	• Enhancing HPC processing capability and diverging into cGMP-compliant manufacturing with creating of novel structures, such as cleanroom facilities, quality systems and quality control in hospital settings

AEMPS, Agencia Española de Medicamentos y Productos Sanitarios; CAR, chimeric antigen receptor; cGMP, Current Good Manufacturing Practice; FACT, Foundation for the Accreditation of Cellular Therapy; FDA, Food and Drug Administration; HPC, Hematopoietic Progenitor Cell; JACIE, Joint Accreditation Committee; POC, point-of-care.

of multiple Prodigy devices in parallel in one grade C facility (ballroom concept) [153]. This centralized cell factory setting permitted the testing of multiple devices in parallel and confirmed the use of nine and seven different devices simultaneously for production of CD20 CAR T and CD19 CAR T, respectively. Importantly, these manufacturing runs resulted in the generation of consistent products [153]. Miltenyi has also successfully performed a comparability run between two centralized "Prodigy cell factories" in Germany and the USA [153]. These validations served as a prerequisite for the clinical trials.

The ultimate test for the Prodigy as a solution for POC manufacturing is a clinical trial in which multiple hospitals use it to produce the same ATMP following a common protocol with centralized coordination and quality assurance. The expected outcome of such a trial would be the successful generation of consistent investigational products across multiple sites. The first such clinical trial (Eudra ID: 2017-002848-32, NCT: NCT03853616) sets to test the Prodigy system at multiple POC sites manufacturing CAR T cells against CD19 (MB-CART19.1) for the treatment of B-cell hematological malignancies and involves seven clinical and four manufacturing sites in Germany [153].

2021 was a historic year for POC manufacturing of ATMPs because the Spanish regulator Agencia Española de Medicamentos y Productos Sanitarios (AEMPS) approved the clinical use of POC manufacturing of a CAR T-cell product under Hospital Exemption at the Hospital Clínic Barcelona [154]. This CAR T-cell product is to be manufactured by the hospital in the Prodigy system, although at a single hospital, this regulatory approval sets the first precedent for manufacturing a marketed ATMP at POC.

Despite successful development and market penetration, the Prodigy may not be perfect and a universal solution for different processes. Ideally, several "GMP-in-the-box" platforms like the Prodigy will be available at POC to allow flexibility based on the process, "back-up" options and local regulatory requirements. A new bioreactor, Cocoon, developed by Octane (acquired and marketed by Lonza), combines several unit operations in one automated device. Cocoon is integrated multifunctional automated device that can be stacked vertically (called the Cocoon Tree) to enable manufacturing scale out of autologous ATMPs. Magnetic cell separation functionality was added recently to the Cocoon [155], and a nucleofector electroporation device (Lonza) could also be added as a module to the Cocoon for cell transfection applications. Launched last year, the ADVA X^3 (by Adva Biotechnology) is aimed for autologous cell products manufacturing specifically at POC. The X^3 system is a fully automated multifunctional device enabled by machine learning/artificial inelegance platform, electronic batch record and remote access.

With the success of CAR T-cell therapies, maturation of product pipelines and significant investment over the last 5–7 years, we are observing a massive development of new tools and technologies, enabling advance manufacturing of cell therapy products. Many of these tools were developed specifically for a modular manufacturing approach (end-to-end) at POC. A variety of available tools allow comparisons at each unit operation in process development and optimization. Availability of multiple tools for each unit operation is also important for mitigating the risk of reliance on a single supply source. Table 4 illustrates the difference in the availability of technological tools (change in technological readiness) in the past 7–8 years. Most of these manufacturing tools were developed with specific applicability at POC.

We are also currently witnessing significant innovations and new paradigms in building and designing of facilities for manufacturing of ATMPs at POC. Once we will get close to commercial manufacturing of ATMPs at POC, the problem of facility capacity planning could be one of the toughest to overcome. Constructing new facilities in a shell space, prefabrication and a modular approach will allow the addition or reduction of cleanrooms/functional modules and, overall, plan capacity according the needs. Currently, several companies on a market offer modular and flexible approach to GMP facility design, which will be especially applicable at POC (see Table 3). Another approach to facility design in the hospital is a ballroom concept hosting multiple fully closed automated devices with individual patient-specific process in one cleanroom (grade C/D, class ISO-7/8). Multiple devices could be places in a ballroom in parallel (Prodigy cell factory) or stacked vertically (Cocoon Tree, OriBiotech bioreactor system). Some developers envision the future of GMP cell therapy facility installation as the "factory-in-a-box" concept. The Cellares Cell Shuttle concept is an automated, flexible, scalable and closed end-to-end robotized manufacturing platform enclosed in a single room (module), which could be easily installed in the hospital. All these innovative approaches to facilities design allow rapid facility installation, validation, flexible use, highly efficient use and capacity planning.

Institutional readiness

The biggest challenges for implementation of POC model at the hospital level are consistent manufacturability, traceability, scalability and quality assurance compliant with cGMP regulations. Readiness of the hospitals for ATMP manufacturing and delivery is frequently ignored in the TRL assessment of decentralized manufacturing (reviewed in [156]). Currently ATMP manufacturing requires a cGMP facility, and many hospitals currently do not have this type of infrastructure. In the last several years, however, we have seen significant changes in the preparedness of academic hospitals as they invest in the deployment of novel cell-gene therapies, much of which can be attributed to the successful commercialization and marketing of ATMPs by the industry [157]. Companies are now starting to interact with hospitals very early in order to understand the risks, resources and infrastructure and to ensure smooth delivery of centrally produced ATMP at POC or to license academically-developed product-candidates. Hospitals, on the other hand, are learning a great deal about GMP regulatory requirements and how to improve their operations [158]. For example, in the case of many autologous ATMPs, hospitals act as vendors in the critical supply chain process, as they supply the apheresis starting material to the pharmaceutical

Table 4
Examples of cell and gene therapy technologies developed in the last 7 years.

Enabling technologies	Examples	
	Before	Now (in the last 7 years)
PBMC/MNC separation, RBC debulking	Ficoll (manual); Cobe 2991 (Terumo); Elutra (Terumo)	Elutra (Terumo); X-LAB (Thermogenesis); Sepax C-Pro (Cytiva Lifesciences); CliniMACS Prodigy (Miltenyi Biotec); Sefia (Cytiva Lifesciences); PXP System (Thermogenesis); Curate (GPB Scientific), Sorterra (MicroMedicine); Lovo (Fresenius Kabi); Rotea (Thermo Scientific)
Cell selection and sorting	CliniMACS (Miltenyi Biotec); FACSJazz/Influx (BD)	CliniMACS Plus, CliniMACS Prodigy, CliniMACS Quant Tyto (Miltenyi Biotec); WOLF (Nanocellect); GigaSort (Cytonome); FX500 (Sony); X-GRAFFE (Biomagnetic Solutions); Highway-1 (Cellular Highways); X-BACS (Thermogenesis)
Automated cell culture/expansion	WAVE (GE Healthcare)	Xuri (Cytiva Lifesciences); Biostat RM (Sartorius); Quantum (Terumo); Cocoon (Lonza); Adva (Adva Bio); PBS (PBS Biotech); CliniMACS Prodigy (Miltenyi Biotec); GRex (Wilson Wolf)
Cell wash, concentration	Cobe 2991 (Terumo), Cytomate (Baxter)	Sepax C-Pro (Cytiva Lifesciences); Lovo (Fresenius Kabi); ekko (FloDesign Sonic/Millipore Sigma); Rotea (Thermo Fisher); X-WASH (Thermogenesis); Sefia (Cytiva Lifesciences)
T-cell activation system	CD3/CD28 Dynabeads, K562 cell line	CD3/CD28 Dynabeads (Thermo Fisher); TransAct (Miltenyi Biotec); ImmunoCult Activator (StemCell Technologies); GMP Cloudz Human T Cell Activation Kit (Bio-Techne)
Integrating cell therapy software	None	Vineti (Vineti), TrakCel (TrakCel), Chronicle (Cytiva Lifesciences); Title 21 (Title 21Health Solutions); MatchSource (To Be The Match Biotherapies); Biotherapies Lab (WellSky); SkylandPIMS (Skyland Analytics)
Modular facility design and installation	None	Xvivo System (Biospherix Medical); G-CON PODs (G-CON Manufacturing); SlateXpace (CRB); KUBio (Cytiva Lifesciences); BioGO (GermFree)

MNC, mononuclear cells; PBMC, peripheral blood mononuclear cells; RBC, red blood cells.

company manufacturing the ATMP. Therefore, the hospital must be audited by the pharmaceutical company and comply with strict regulations as GMP supply chain.

Besides activities related to deployment of commercial ATMPs, large academic medical centers continue to build their own manufacturing capacity to support investigator-initiated trials. These GMP facilities are often (but not always) created as an extension of stem cell processing laboratories or stand-alone entities. Proliferation of such newly constructed cell therapy GMP facilities in large hospitals is a response to commercial success of certain ATMPs, developed in academia and massive investment from the industry. With the maturation of the field and readiness of POC manufacturing at scale, these facilities could be used for massive deployment of novel regulatory-authorized ATMPs. Further direction may soon be released by FDA in the planned guidance, "Regulation of Human Cells, Tissues, and Cellular Tissue-Based Products (HCT/Ps) – Small Entity Compliance Guide; Guidance for Industry", scheduled for release in calendar year 2022.

Regulatory readiness

Regulators in the USA and European Union are acknowledging the attractiveness of the POC model for ATMP manufacturing and are willing to discuss potential scenarios for authorization of decentralized manufacturing. Section 11.3.3 of the European ATMP GMP Guideline (2017) specifically addresses decentralized manufacturing for "cases where manufacturing of the ATMP needs to take place in sites close to the patient" [159]. The guidance describes the scenario for market-authorized ATMPs, manufacturing of which decentralized to multiple sites. One of the decentralized sites should assume a role of "central site" for oversight of all other sites. The "central site" could be marketing authorization holder.

European Pharmaceutical Inspection Convention has released this year GMP Guide Annex 2A where specific part (section 6.15) is dedicated to "batch release process in cases of decentralised/point-of-care manufacturing." The section defines a "responsible site" and "authorized person" for the case of POC manufacture [160].

The United Kingdom Medicines and Healthcare products Regulatory Agency has released this year a public Consultation on Point of Care Manufacturing. It proposes a couple of concepts, which may support POC manufacture of ATMPs: (i) "hub-and-spoke" model with one licensed control site and multiple satellite hospitals and (ii) POC Master File a key source information to which site will refer [161].

The FDA envisions a potential scenario of issuing multiple biologics license applications for the similar product to individual physicians or small groups of physicians or small firms [152]. The FDA views this innovative pathway as appropriate for cell therapy products for which manufacturing is not highly complex but yet involves more than minimal manipulation processes. One example of such approach is a successful National Institutes of Health–sponsored phase 3 clinical trial of allogeneic human islet transplantation in type 1 diabetes, which was conducted by a consortium of eight academic sites across North America [162]. The manufacturing protocol was harmonized and controlled by a common "master production batch record." Despite the success of this phase 3 trial as academic-based medical innovation, it did not result in multiple biologics license applications, most likely due to the high cost associated with a regulatory filing for licensure. Yet another interesting example is the recent Investigational New Drug clearance of the POC electroporator (UltraPorator) device, developed by Precigen [163]. A similar approach was taken by Lupagen, which is developing a SideCAR T device specifically for CAR T-cell therapies at POC. This could be an example of "simplification of CAR T process," where collected patient's T cells undergo genetic modification in the device and returned back to the patient within 24 hours.

Despite the willingness of regulators to support POC manufacturing of ATMPs, it is still unknown how they will ensure comparability between sites and consistency of the products, across the sites. Of course, another big challenge is process changes and amendments during clinical trials and management of variations post-market authorization. If the model of the central site (hub) would be prevalent in POC manufacturing, what authority will it have? Will the hub hold the license exclusively, testing all products and manage all satellite sites, or will sites have some independence, relying on the reference to regulatory master file?

With success of CAR T-cell therapies and massive investment in the field of cell and gene therapy, we are witnessing a significant advancement in TRL for POC manufacturing of ATMPs. It is especially prominent in enabling technology development, where a number of manufacturing tools specifically designed for or applicable at POC is exploding in the last 5–7 years. The future of ATMP manufacturing at POC could be in the hands of medical device developers.

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In recent years, industry contributed significantly to the readiness level of hospitals for ATMP manufacturing and distribution. As such, the emerging frontiers for POC are likely solutions to the logistical and regulatory issues that currently impede the manufacturing of ATMPs at POC. As these solutions are put in place, the anticipation builds for the first precedent for marketing of the ATMP, manufactured at POC.

Summary

In the past 5 years, significant progress has been made using cell and gene therapy products. However, additional advancements need to be made to increase the durability of response for hematologic cancers, to target solid tumors and to broaden the applicability of these therapies. CAR T cells as a treatment modality have shown the greatest success thus far within B-cell–related hematologic malignancies, and effectively overcome barriers related to human leukocyte antigen restriction and other strategies linked to MHC presentation, facilitating broader use of this immunotherapeutic approach. In contrast, engineered TCR-based strategies offer promise by accessing intracellular targets. However, significant challenges remain both in terms of optimizing the properties intrinsic to the TCR and CAR T-cell product and overcoming tumor immune resistance mechanisms, particularly in solid tumors and other hematologic malignancies beyond B-cell–associated neoplasms. Continued advances will allow a greater proportion of patients with a wider variety of cancers to derive benefit from these novel therapies and achieve long-term survival.

As the clinical use of NK cells continues to expand, the sources of NK cells will continue to be further defined and optimized to support the required specialized manufacturing of the cell products. In addition, the novel engineering of NK cells and manipulation of their metabolic fitness are promising areas of research to improve their efficacy in clinical trials.

While no singular novel treatment will be successful for all cancers, we firmly believe that advances in these first three frontiers will generate therapies that will allow us to realize true clinical benefit for patients in the coming years. Finally, with recent technological advances, we may see a paradigm shift in moving of ATMPs manufacturing from centralized plant to the hospital. TRL assessment is required for better understanding of current state, maturation and future perspective of ATMP manufacturing at POC. These advances will ensure once novel cellular therapies finally do unlock successful treatment of currently incurable cancers that these treatments will be widely available.

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Declaration of Competing Interest

P.J.H. is a co-founder, consultant and serves on the board of directors of Mana Therapeutics. He is on the scientific advisory boards of Cellevolve, Cellenkos, Discovery Life Sciences and MicroFluidx and has consulted for Maxcyte.

Author Contributions

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