

EXPERT INSIGHT

Unlocking the therapeutic and commercial potential of CAR-T technology

Michelle J Smith, Brittni M Peterson & Barbara A Nelsen

The striking clinical results of CAR-T therapies in blood cancers have shifted the cell therapy field from one that has future potential, to one offering hope of a future to those living with cancer. Since 2012 the field has expanded beyond investigators to investment and initial public offerings, individual 'cures' and industry growth. Here, we review the current state of CAR-T therapeutics, from improvements in CAR-T technology to clinical results, business growth and future applications. Finally, we discuss the remaining barriers to successful commercialization of CAR-T technology identified by key investors within this rapidly expanding field.

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The body's adaptive immune system provides a powerful and safe way to eliminate health threats. Beginning with vaccines in the late 1700s and moving to the use of antisera, monoclonal antibodies and most recently T cells, researchers have sought to harness people's own immune systems to fight their own diseases. Today, technology has enabled the genetic modification of T cells to express a chimeric antigen

receptor (CAR) that allows them to recognize antigens on malignant cells. This modification eliminates the need for other components of the immune system to present the antigen to the T cell. In turn, this streamlines the process of triggering the cytolytic signaling cascade, making these T cells cancer-killing machines.

In the past few years, CAR-T cells have proven to be a highly

effective therapeutic tool in treating certain types of cancers, particularly hematological malignancies. The clinical successes achieved by CAR-T therapies have stimulated interest and participation from industry players. Millions of dollars from private, public and government sectors have been invested in the last few years and collaborations among bio-ventures and academic facilities have been initiated. The

increased activity has translated to the optimization of CAR-T constructs at the bench, and the development of applications both within and beyond hematological cancers at the bedside. However, more improvements are required to increase the safety and efficacy of these therapeutics and the advance towards their commercialization. In this article, we review the scientific and clinical evolution of CAR-T cells, their translation into treatments for patients and the commercialization barriers that need to be overcome to unlock the potential of CAR-T therapies.

THE BUSINESS OF CAR-T THERAPEUTICS

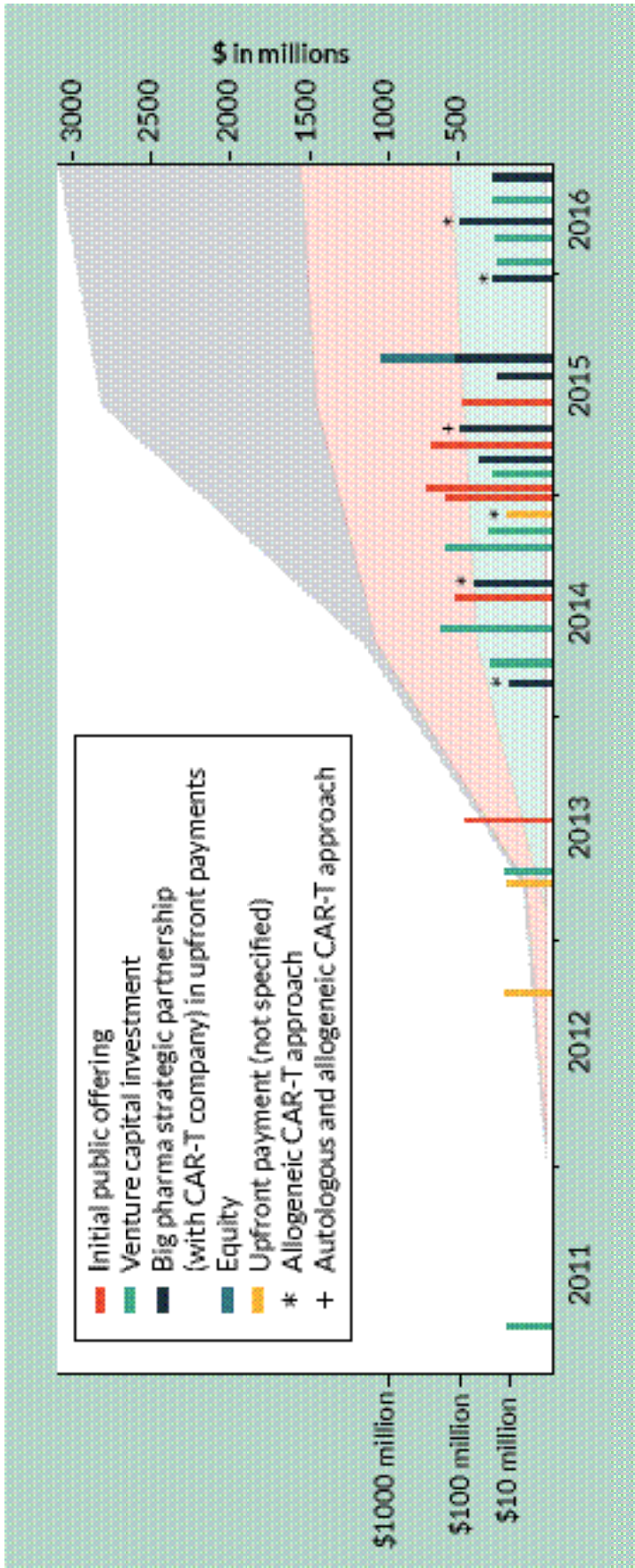
The development of CAR-T cells by Zelig Eshhar and Steven Rosenberg in 1990 was an attempt to improve our ability to use the body's innate immune system to target and 'cure' cancer [1]. More than two decades later, this approach has reached a tipping point. The striking clinical success achieved in treating acute lymphoblastic leukemia (ALL) in the case of Emily Whitehead, the first pediatric patient to be treated with CAR-T therapy in spring 2012, was a game changer. It was this clinical result and the duration of its response that engaged the interest of investors, who have contributed \$600 million in venture capital (VC) to directly support the development of CAR-T therapeutics through August 2016 (Figure 1 & Table 1) [Nelsen Biomedical Analysis; 2]. The majority of this has been invested since 2012 and dedicated to autologous CAR-T approaches. Within a few years of gaining commitments from the VC community,

these companies garnered interest from the public markets, with initial public offerings totaling nearly \$1 billion to date (Figure 1 & Table 2) [Nelsen Biomedical Analysis].

Big Pharma's hesitation to engage in the cell therapy industry also shifted in 2012, with the clinical results of CAR-T in ALL and chronic lymphocytic leukemia (CLL). That year marked the first commercial partnership in this space, between Novartis and the University of Pennsylvania, USA (Figure 1 & Table 3) [3]. Since 2012, seven other Big Pharma companies have placed bets in the CAR-T space, totaling at least \$1.5 billion in upfront and equity payments and over \$2 billion in additional milestone, royalty and other payments (Figure 1 & Table 3). It is worth noting, however, that for most Big Pharma companies their focus is on allogeneic approaches. Carolyn Green, Executive Director of Strategic Investments at Pfizer, has commented, "Autologous approaches do not fit the business model of Pfizer. CAR-T therapy will require very different manufacturing and distribution than traditional pharmaceuticals or antibodies – which are 'one-to-many' therapies. Pfizer has only one focus on CAR-T and that is the allogeneic approach, where an off-the-shelf product can be given as a 'one-to-many' strategy. We partnered with Cellectis on this." In the past 2 years, three of the top 15 highest valued immuno-oncology partnerships were Big Pharma's investments in CAR-T therapies [4]. Interestingly, two of these three partnerships are focused on allogeneic approaches and each is worth at least twice as much as the autologous partnership [4].

Aside from the VC and Big Pharma investment deals discussed

FIGURE 1
CAR-T companies: venture investments, initial public offerings and pharmaceutical partnerships.



Beginning in 2011, with Kite Pharma, venture capitalists have invested in six companies developing CAR-T therapeutics. Total venture capital (VC) dollars have reached over \$600 million as of September 1st 2016 (see [Table 1](#) for details). The six companies funded, the majority of which are developing autologous CAR-T therapies, completed initial public offerings totaling nearly \$1 billion (see [Table 2](#) for details). Since the first Big Pharma strategic partnership in the CAR-T space between Novartis and the University of Pennsylvania in 2012, seven other Big Pharma companies have followed suit, placing bets of at least \$1.5 billion in upfront payments, the majority for allogeneic approaches marked by asterisks (see [Table 3](#) for details). Includes only VC funding for companies involved in CAR-T program(s) at the time of investment. For example, VC funding of Bluebird Bio occurred prior to their CAR-T programs, while the company had only a gene therapy focus. These investments are not included. See [Table 1](#) for details.

Includes only initial public offerings where the company had a CAR-T focus at the time of going public. See [Table 2](#) for details.

Includes only strategic partnerships where Big Pharma companies invest in and obtain rights to CAR-T therapeutic programs. Not included, for example, is the Roche and Genentech partnership with Kite Pharma in March 2016 to combine Kite's CAR-T technology with Roche or Genentech's small molecules. See Supplementary Table 1 for other deals like this. Payments are upfront only; see [Table 3](#) for additional details on milestone, royalties and other terms.

Sources: Company press releases, Nelsen Biomedical Analysis.

► **TABLE 1****Venture capital investment in CAR-T companies 2011–2016.**

Company	Venture capital (\$ in millions)	Date	CAR-T approach
Kite Pharma	15	Mar 2011	Autologous
Kite Pharma	20	May 2013	Autologous
Kite Pharma	50	Apr 2014	Autologous
Juno	176	Apr 2014	Autologous
Juno	134	Aug 2014	Autologous
Bellicum	55	Aug 2014	Autologous
Autolus	45	Jan 2015	Autologous
Poseida	23	Dec 2015	Allogeneic
CARsgen	30	Jan 2016	Autologous
Autolus	57	Mar 2016	Autologous
Total	605		

Includes only venture capital funding for companies involved in CAR-T program(s) at the time of investment. For example, venture capital funding of Bluebird Bio occurred prior to their CAR-T programs, while the company had only a gene therapy focus. These investments are not included.

Source: Company press releases.

above, there have been over 75 additional partnership, acquisition and licensing deals done from 2012 through August 2016 worth at least another \$650 million in disclosed upfront payments and \$2 billion in additional milestone, royalty and other payments [Nelsen Biomedical Analysis]. The number of deals in this space has grown every year, from just three deals in 2012 to 35 in 2015. With already 26 deals as of September 1, 2016, the full-year 2016 numbers will likely reach at least the 2015 figure. These deals cover intellectual property, new approaches for creating CARs using gene-editing technologies, expansion to allogeneic approaches, combination therapies and more (Supplementary Table 1, nelsenbiomedical.com/cartdeals).

Overall, the T-cell immunotherapy market is projected to be worth \$30 billion by 2030, with CAR-T therapies likely to garner the most attention in the near future [5]. To understand the true commercial

outlook it is necessary to examine the scientific evolution of CAR-Ts, their clinical applications and the barriers to commercialization.

THE EVOLUTION OF CAR-Ts

Monoclonal antibody-based therapies achieve anti-tumor activity by influencing T cells within the patient's body through cell-intrinsic signaling. This is one way to modify the adaptive immune system and target cancer cells. At the National Institutes of Health, USA, Steven Rosenberg took a different approach by harvesting, expanding and re-infusing tumor-infiltrating lymphocytes from melanoma patients. While this technique laid the groundwork for future T-cell-based therapies, it showed limited efficacy. In addition, when the affinity of the T-cell receptor (TCR) was increased, safety issues emerged [6,7]. This therapy, like all based on TCRs,

► **TABLE 2**
Initial public offerings of CAR-T companies 2011–2016.

Company	\$ IPO (in millions)	IPO date	CAR-T approach
Bluebird bio	101	Jun 2013	Autologous
Kite	128	Jun 2014	Autologous
Bellicum	140	Dec 2014	Autologous
Juno	265	Dec 2014	Autologous
Cellectis	228	Mar 2015	Allogeneic
Celyad	100	May 2015	Allogeneic and autologous
Total	962		

Only includes IPOs where the company had a CAR-T focus at the time of going public.
IPO: Initial public offering. Source: Company press releases.

was dependent on the presentation of its target antigen in the context of a major histocompatibility molecule (MHC), which increases the complexity of engineering the TCR. The development of the CAR-T cells addressed this shortcoming of TCR-engineered T cells. The CAR combines the antigen-recognition portion of the B-cell receptor, which functions independently of MHC, with the T-cell intracellular signaling domain (CD3 ζ) [8]. This allows the T cell to recognize any surface molecule to which an antibody can be made and results in a highly effective therapeutic tool, even in disease refractory to chemotherapy.

First-, second- & third-generation CAR-Ts: focus on improving effectiveness

In the most basic CAR construct, the functional sections are the variable portion of an antibody that is specific for the intended target (scFv or antigen recognition), usually a tumor-associated antigen, and the CD3 ζ [9]. When the scFv binds to its target antigen, the CD3 ζ initiates the TCR signaling cascade, thus activating the cytolytic function of the CAR-T [10]. The first-generation CARs (Figure 2) provide only

one signal of the three required for activation of T cells [11,12]. As a result, T cells receiving stimulation through a first-generation CAR without accompanying co-stimulation often become anergic. This negatively impacts the ability of the cell to function effectively and its ability to persist over time.

These limitations were addressed by incorporating the signaling domain of a co-stimulatory molecule into the CAR construct (Figure 2). This iteration created the second-generation of CARs. The co-stimulatory domains they include vary, but are most commonly derived from CD27, CD28, OX40 or 4-1BB. The inclusion of one of these co-stimulatory domains significantly improves the expansion and persistence of CAR-T cells, thereby improving their anti-tumor effect [10,13]. Following the success of the second-generation of CAR-T technology, more than one co-stimulatory molecule was added in the hopes of achieving an additive or synergistic increase in effectiveness (Figure 2). However, this did not prove out, with the third-generation of CAR-Ts showing no improvement in tumor clearance. In fact, in the majority of cases second-generation CAR-Ts

► **TABLE 3****Big Pharma–CAR-T companies strategic partnerships 2011–2016.**

Company/ Institution	Big Pharma	Deal type	Deal value	CAR-T approach	Date
University of Pennsylvania	Novartis	Exclusive global research and licensing agreement	Not disclosed	Autologous	Aug 2012
Bluebird Bio	Celgene	Exclusive multiyear research and collabo- ration agreement and license agreement	Not disclosed	Autologous	Mar 2013
Cellectis	Servier	Collaboration	Servier will pay \$10 million upfront and up to \$140 million for each of the 6 product can- didates potentially developed	Allogeneic	Feb 2014
Cellectis	Pfizer	Collaboration	Pfizer will pay \$80 million upfront plus up to \$185 million per product and royalties	Allogeneic	Jun 2014
Transposagen	John- son and Johnson	Collaboration and license agreement	Unspecified upfront; Johnson and Johnson will pay up to \$292 million per treatment in milestones	Allogeneic	Nov 2014
Kite Pharma	Amgen	Collaboration	Amgen to pay \$60 million upfront and up to \$525 million per product in milestone pay- ments, plus royalties on sales and IP licensing	Autologous	Jan 2015
Ziopharm/ Intrexon	Merck	Strategic collaboration and license agreement	Merck to pay \$115 million upfront, fee will be split equally between Intrexon and partner Ziopharm Oncology along with a commitment of up to \$826 million more in milestones for the first two programs	Autologous & allogeneic	Mar 2015
Bluebird Bio	Celgene	Collaboration	Celgene to pay \$25 million	Autologous	Jun 2015
Juno	Celgene	Collaboration	Celgene to pay approximately \$1 billion, composed of an approximately \$150 million upfront payment and approx- imately \$849.8 million to purchase 9,137,672 shares of Juno's common stock at \$93.00 per share	Autologous	Jun 2015
Cellectis	Servier	Exclusive global license and collaboration agreement	Servier to pay \$38.2 million upon signature and eligible for over \$300 million of milestone payments, R&D financing and royalties	Allogeneic	Nov 2015
Precision Biosciences	Baxalta	Collaboration	Baxalta to pay Precision \$105 million upfront, followed by up to \$1.6 billion in option fees and payments milestones and royalties	Allogeneic	Feb 2016
Juno	Celgene	Exercised option	Celgene will pay Juno a fee of \$50 million and the companies will now share global develop- ment expenses for products in the CD19 program	Autologous	Apr 2016

Only includes strategic partnerships where Big Pharma companies invest in and obtain rights to CAR-T therapeutic programs. Not included, for example, the Roche and Genentech partnership with Kite Pharma in March 2016 to combine Kite's CAR-T technology with Roche or Genentech's small molecules. See Supplementary Table 1 for other deals like this. Payments are upfront only, Source: Company press releases.

have achieved better outcomes [14] and these, but not third-generation CAR-Ts, have proceeded into clinical trials.

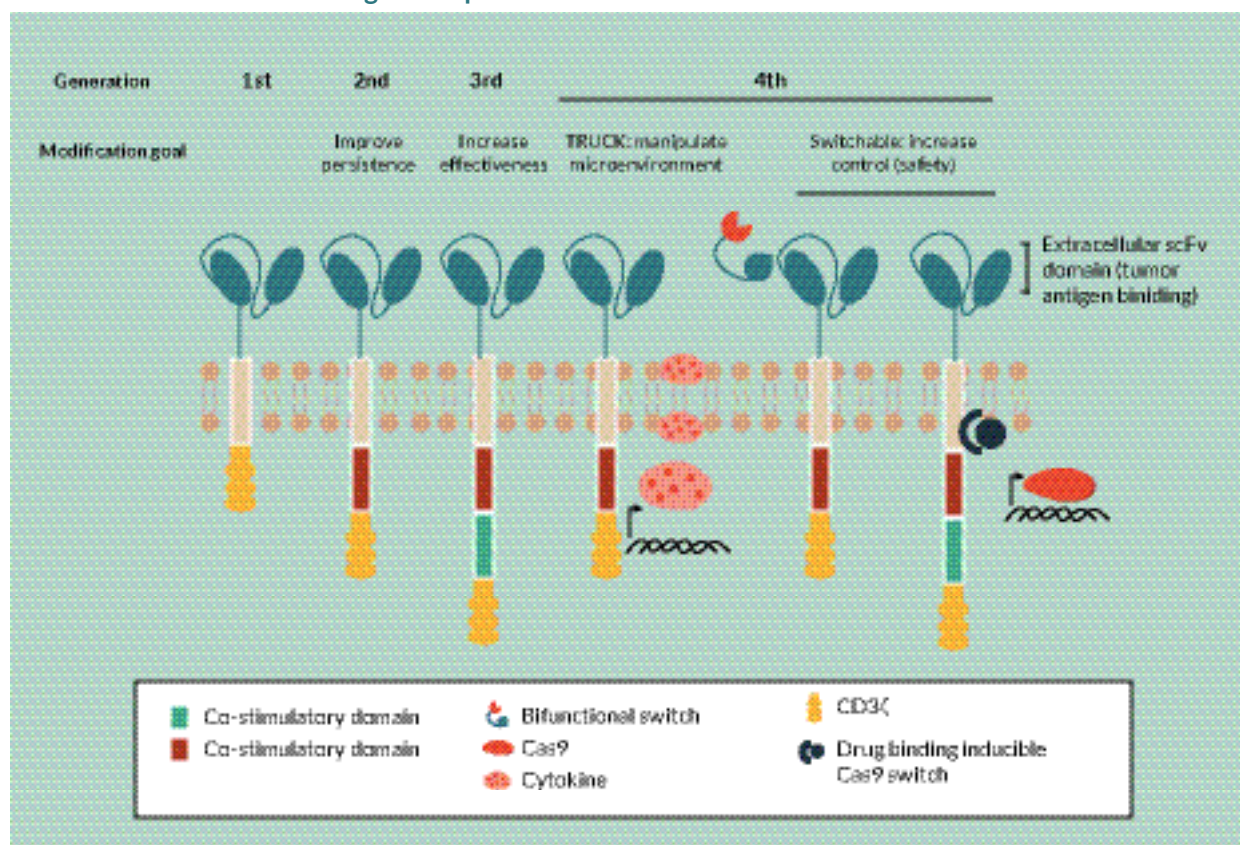
Next-generation CARs: to overcome suppression & improve safety

While second-generation CARs have achieved some significant pre-clinical and clinical successes, more improvements in safety and efficacy are needed. For example, even in the most successful CAR-T target to date, anti-CD19 CAR-T therapies, responses are variable. These therapeutics are more effective in treating

CD19-expressing ALL than in other applications [15–18]. Reduced efficacy in some applications is due in part to suppressive effects that the tumor has on the CAR-T engineered cells. And these suppressive effects of the tumor microenvironment are more pronounced in solid tumors than in hematopoietic tumors. With the aim of correcting this suppressive effect, a new generation of CAR-T referred to as TRUCKs, deliver a ‘payload’ of immune-activating chemokines or cytokines (Figure 2). This enhances the effectiveness of the CAR-T through recruitment of additional immune cells to the tumor

► FIGURE 2

The evolution of chimeric antigen receptors.



The first-generation CARs include CD3ζ, one of the three signals required for the activation of T cells. Second-generation CARs incorporate a second, co-stimulatory signaling domain to improve the persistence of CAR-T cells. In third-generation CARs, more than one co-stimulatory molecule was added, but a resultant increase in effectiveness has yet to be conclusively proven. There are several different iterations considered to be fourth generation. One of which are TRUCKs that deliver immune activating chemokines or cytokines to manipulate the microenvironment. The other two are switchable CARs that can be regulated by a bi-functional antibody to control activation or by small molecules that can induce apoptosis, both of which are aimed at increasing safety.

Sources: [9,10,13,20,22,23,24].

microenvironment and increased tumor killing via both these additional cells and the inflammatory cytokines themselves [19,20].

Regulatable CARs focus on improved safety

Many patients, approximately 55%, experience side effects related to cytokine release as a result of CAR-T therapy [21]. Cytokine release syndrome side effects are often mild, flu-like symptoms but can be severe, including hypotension, vascular leak, pulmonary edema and coagulopathy, leading to multi-organ failure and even death. Currently these complications are managed with corticosteroids and anti-IL-6 therapy [15]. Investigators are experimenting with incorporating elements into new CAR designs to allow them to be regulated, with the aim of increasing the safety of these therapies. Examples of recently developed safety measures include switchable CAR-T whose activity can be modulated by small molecules, and the inclusion of an inducible caspase 9 to allow directed apoptosis of the CAR-T (Figure 2) [22,23]. Additional approaches continue to be developed with the hope of improving safety through alternatives such as antibody-coupled T-cell receptor technology, in which the receptor on the T cell must be activated by a separately administered antibody and in this way titration of response can be achieved.

CLINICAL APPLICATIONS

The optimization of CAR-T constructs at the bench has translated not only to the success of these therapies in ALL, but also to the expansion of CAR-T clinical trials. From 2011 to 2015, trials using CAR-T

therapies have grown in tandem with cellular immunotherapy, comprising 50% of all new cellular immunotherapy clinical trials and 25% of total cell therapy clinical trials overall (Figure 3A) [Nelsen Biomedical Analysis]. Growth has occurred in both the total number of new trials started each year and also progression to later stages (Figure 3B). As of September 1, 2016, the number of new CAR-T cell therapy trials initiated has already surpassed the 2015 figure (Figure 3B) [Nelsen Biomedical Analysis].

Blood cancers

CAR-T cells have shown extraordinary success in treating B-cell hematologic malignancies such as ALL, CLL and non-hodgkins lymphoma (NHL). Depending on the specific trial and indication targeted, overall response rates typically range from 40 to 94% [21]. Collectively, recent trials in patients with ALL, CLL or NHL show that on average 75% of patients experience a complete or partial response, with a majority (67%) showing a complete response [21].

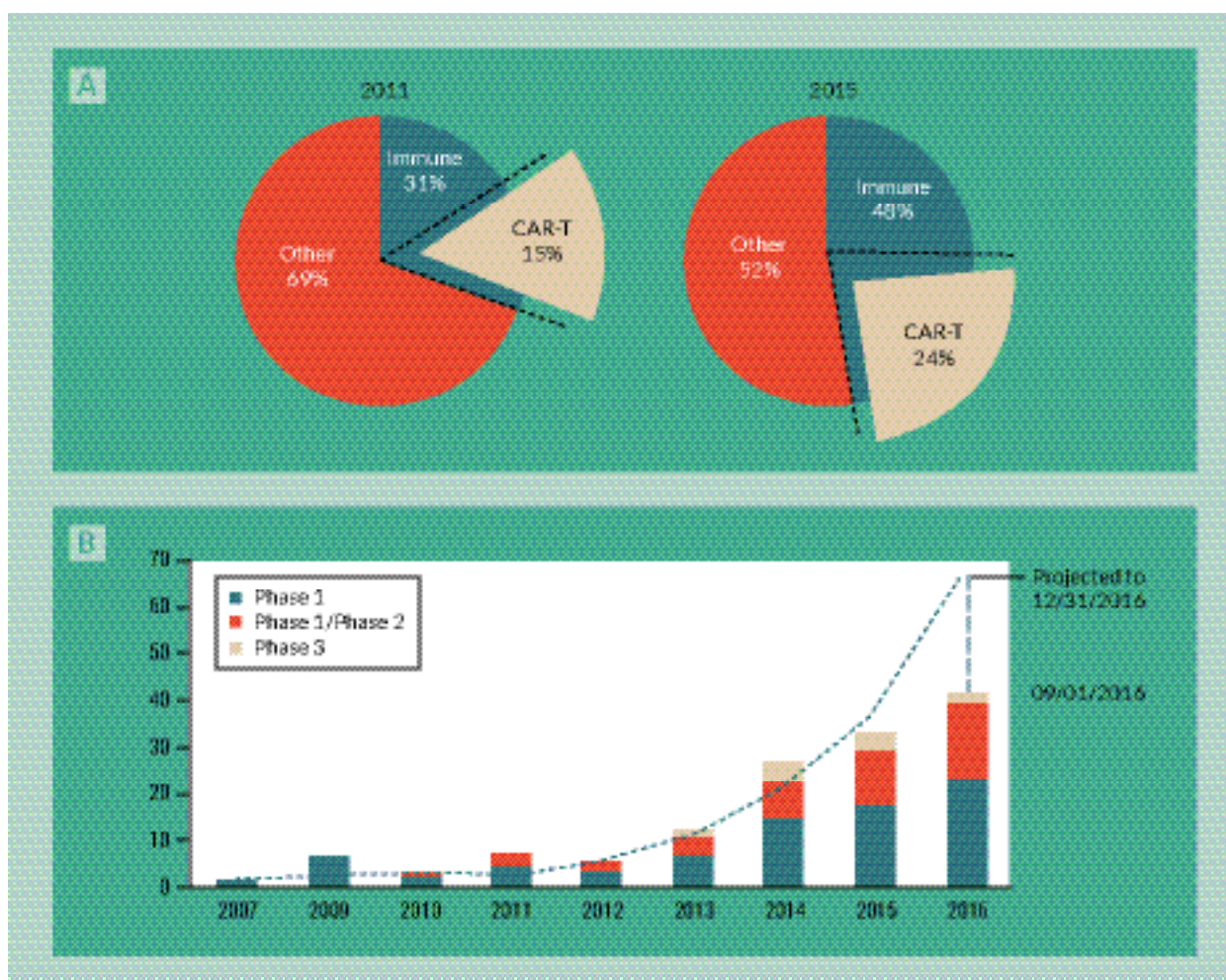
Of these three blood cancers, the results in patients with refractory or relapsed ALL have been the most impressive. Most people have heard of the poster child of this game changer in the fight against this deadly disease, Emily Whitehead whose last ditch treatment by Dr Porter at the University of Pennsylvania has left the now 11-year-old cancer free for over 4 years. Since then, ALL patients treated with CAR-T therapies targeting CD19 exhibit complete response rates of approximately 90% [24], a remarkable improvement over other existing therapies for ALL, such as chemotherapy and allo-stem cell transplant (SCT) where response

rates and durable remissions are <50% [25]. Notably, this 90% complete response success rate has been observed across all age groups, from pediatric to adult populations, and across independent trials taking place at different academic medical facilities [21,24,25]. The consistency in these successful outcomes in blood cancers is particularly profound, given that inconsistencies tend to arise due to the variety of protocols, infrastructure and clinical staff used across the clinical trials.

Other CAR-T therapies targeting CD19 to treat B-cell hematologic malignancies are equally impressive compared to response rates with current therapeutics. However, in comparison with ALL, CLL and NHL have yielded more variable response rates – between 40 and 85% overall response and 20 and 60% complete response [21,25]. While ongoing research is focused on understanding how to achieve more effective and consistent results using CAR-Ts for CLL and

► FIGURE 3

CAR-T clinical trials.



(A) CAR-T clinical trials as a percent of all cell therapy trials. From 2011 to 2015, trials using CAR-T therapies have grown in tandem with cellular immunotherapy, comprising 50% of all new cellular immunotherapy clinical trials and 25% of total cell therapy clinical trials overall. (B) Since 2007 the number of new CAR-T therapy trials started each year has climbed dramatically. With the total already at 41 as of September 1 2016, this number could reach nearly 70 by the end of the 2016 calendar year. Increases in overall numbers of CAR-T trials are matched by progression through phases of development from Phase 1 to Phase 2.

Source: Nelsen Biomedical Analysis.

NHL, nevertheless this therapeutic approach for these malignancies appears to be more promising than those that are currently available.

Expansion to solid tumors

Driven by the success of the CD19-CAR in treating ALL, CLL and NHL, CARs specific for other cell-surface markers are being explored for the treatment of many different types of malignancies. While some target hematologic malignancies, such as the anti-CD38 CAR [26–28], others are focused on solid tumors. Examples include T cells expressing CARs specific for IL-11R α and variants of CD44, which are targeted for head and neck, pancreatic, gastric, breast, cervical and colon cancers [29–31], or a CSPG4-CAR, which targets melanoma, triple-negative breast cancer, glioblastoma multiforme, mesothelioma, head and neck cancers, and osteosarcomas [32–34]. Clinical trials are ongoing for the treatment of neuroblastomas and osteosarcomas with a GD2-CAR-T therapy [35]. For neuroblastoma, early results look promising for the only program beyond blood cancers that has progressed to Phase 2 clinical trials (NCT02765243) [35]. Additional antigens are being tested preclinically for targeting solid tumors. Brain cancers, CNS cancers, gliomas, glioblastoma multiforme, and head and neck cancers are being targeted with an ErbB2-CAR-T cell [36]. Breast cancer responsiveness to CAR-T therapy is being explored using c-MET and carcinoembryonic antigen (CEA) CAR-T cells [37], while prostate cancers are being targeted with a prostate-specific membrane antigen CAR-T construct [38].

What's the real potential for CAR-T in cancers beyond blood? One investor noted that: "We know

that they work for B-cell lymphomas really well, but there's going to need to be a suite of technical alterations for it to work in various other applications." (Ben Auspitz, F-Prime Capital) Some barriers to success in solid tumors that need to be addressed through more research and technical improvements include:

- ▶ Identification of safe and effective antigenic targets for each tumor type
- ▶ Efficient localization of CAR-T cells within the tumor
- ▶ Neutralization of the immunosuppressive tumor microenvironment

Research to address these barriers is ongoing and varies by tumor type, currently ranging from early pre-clinical studies to clinical trials. As we discussed earlier, many antigenic targets are under investigation to expand CAR-T applications to other blood cancers and solid tumors. But most of these antigens are expressed on a variety of normal cell types. In solid tumors, targeting CARs to overexpressed antigens can lead to the destruction of healthy solid organ tissue, such as in the case of the off target-mediated mortality seen in HER2 CAR-T therapy for colon cancer [39]. So, clearly, safety is a concern and solid tumor-associated antigens must be carefully selected. A variety of approaches for optimizing CAR-T therapy for solid tumors range from directly administering CAR-T cells to the tumor, to combining CAR-T with small molecules that regulate cytokine and surface receptor responses, to transient CAR expression. These strategies are thoroughly summarized in two recent reviews in *Molecular Therapy – Oncolytics* [40] and the *Journal of Cytotherapy* [41].

CAR-T therapies beyond cancer

Further on the horizon, researchers have begun work to adapt CAR technology for the treatment of autoimmune diseases. For these applications the CAR is engineered to be reactive against self-antigens, but instead of being attached to a conventional T cell, it is attached to a regulatory T cell (Treg). In this way, it is hoped that tolerance against specific self-antigens can be achieved. This method has achieved some pre-clinical successes in mouse models of colitis and multiple sclerosis. CAR-Tregs for the treatment of colitis have been generated against multiple targets (2,4,6-trinitrophenol and CEA); both have shown specificity, improvement in survival and amelioration of inflammation-associated symptoms [42,43]. In the treatment of experimental autoimmune encephalomyelitis, which is a mouse model for multiple sclerosis, the CAR used was directed against myelin oligodendrocyte glycoprotein and produced a durable reduction in symptoms, even in the face of a second induction of disease [44].

Clearly, CAR-T technology holds promise in a variety of therapeutic areas when and where the highly specific nature of its antigen targeting can be leveraged. However, investors we spoke with said that only when additional antigens can be specifically targeted and show efficacy similar to that achieved with CD19 and B-cell lymphoma, will the field beyond blood cancers take off.

CHALLENGES FOR CAR-T

There remain technical, clinical and commercial challenges that must be overcome for the

widespread adoption and production of CAR-T therapy.

Technical challenges

Technically, some areas requiring additional work include:

- ▶ Identification of safe and effective antigenic targets for a variety of tumor types
- ▶ Optimizing CAR design for maximum efficacy
- ▶ Inclusion of appropriate safety controls into CAR design

These topics have already been discussed above. Briefly, to the first point, identifying tumor-specific antigens (not expressed by normal cells) will be key to avoiding on-target, off-tumor effects. However, even with effective antigens to direct CARs against, the tumor can down-regulate the antigen (antigenic escape), which can be detrimental to therapeutic efficacy. The prevailing approach to combatting antigenic escape is to target multiple antigens on the tumor, such as using CD22-CAR-T cells to treat patients who have relapsed with CD19-negative ALL following successful CD19-CAR-T therapy (clinical trials NCT02650414 and NCT02315612).

Often overlooked, but equally important to efficacy is how the cell therapy product is manufactured. Recent work has shown that “the method used for expanding T cells prior to infusion is an essential determinant of their *in vivo* efficacy” [45]. Companies need to give serious considerations to the way in which CAR-T cells are generated, as this can have significant impacts on efficacy. With manufacturing, enrichment of the ‘active ingredient’ may be achieved through a combination of:

- ▶ Improved protocols for transduction efficiency and cell selection

- ▶ Optimizing *in vitro* growth conditions to enhance outgrowth of the desired cell population
- ▶ Enrichment of the 'active ingredient'

The effectiveness of CAR-T therapy may reasonably be expected to depend on the percentage of cells expressing the CAR of interest. In fact, comparing three different protocols for manufacturing CD19-CAR-T cells for clinical trials in ALL demonstrates the significant impact that the transduction efficiency can make in the results. Cells produced with one manufacturing protocol (see the first column in **Table 4**) [45] did not yield any responses during a Phase 1 clinical trial. Modifying both the vector and the transduction and expansion steps in a different manufacturing protocol improved efficacy significantly, giving a clinical response rate of 74%

(see the second column in **Table 4**) [46]. In a third Phase I clinical trial for B-NHL, CAR-T cells produced from a central memory cell population using a more complex protocol achieved an 88% response rate. This was an improvement on a similar simpler protocol that utilized a bulk T-cell starting population and resulted in only a 74% response rate for B-ALL, which traditionally is the most responsive to CD19 CAR-T therapy (see column 3 in **Table 4**) [47,48]. Through these and other examples, the importance of optimizing transduction efficiency during manufacturing is clear. Accordingly, clinical protocols that do not include enrichment for cells expressing the target CAR may require higher initial transduction efficiencies. In addition, shifting manufacturing processes to optimize the input population of T cells

► **TABLE 4** — Comparison of selected CAR-T manufacturing protocols.

		Protocol 1	Protocol 2	Protocol 3
Step 1	Cancer Type	CD19+ ALL and CLL	CD19+ ALL	CD19+ NHL
	Patient Population	Adult	Pediatric and Young Adult	Adult
	Cell Enrichment	All T cells	All T cells	Memory T Cells
Step 2	Transduction	Spinnoculation with Retronectin	Retronectin (No Spinnoculation)	Spinnoculation with Retronectin
Step 3	Growth Factors	IL-2	IL-2	IL-2, IL-15
Step 4	Initial Expansion Step	Yes	Yes	Yes
Step 5	Stimulation Bead Removal	Yes	Yes	Yes
Step 6	Second Expansion Step	No	Yes	Yes
	Vector	SFG (Retrovirus)	MSGV (Retrovirus)	HIV (Lentivirus)
	% CAR Expression/Product	14–40%	50–80%	60–90%
	Total Cell Fold Expansion	100–700x	10x	600x
	Total Time Required	2–3 weeks	1–2 weeks	3–6 weeks
	Clinical Results	NR	74% RR	88% RR

with memory subsets, which have shown increased effectiveness [49], may further improve success rates.

Optimizing growth conditions *in vitro* to improve results *in vivo*

A second area of focus should be the specific media, cytokines and atmospheric conditions provided during manufacturing. Small perturbations in these conditions can strongly influence the outgrowth of subpopulations within a heterogeneous input population. Optimizing the culture conditions to produce the required population through pre-culture enrichment protocols and control of media and atmospheric conditions will be an advantage in the production of adoptive cell therapies. Consider, for example, differing effects that co-stimulatory molecules may have on the metabolic needs of CAR-T cells. If the co-stimulatory molecule is CD28, the cell is pushed towards glycolysis and will require higher glucose levels. If the co-stimulatory molecule is 4-1BB, the cell is pushed towards oxidative phosphorylation and will require higher oxygen levels [50]. These are important considerations for both therapeutic design and manufacturing. Oxidative phosphorylation is more energetically efficient, so, for a hematopoietic target, 4-1BB may be the obvious choice for co-stimulatory molecule. However, for solid tumors existing in a hypoxic microenvironment, relying more on glycolysis through CD28 could be an advantage as it reduces the requirement for oxygen. When manufacturing these cells, yields of therapeutically effective CAR-Ts may be improved through

careful monitoring and modification of oxygen and glucose levels throughout manufacturing.

Clinical challenges

Clinical challenges also pose a barrier to the successful commercialization of CAR-T. Three areas of particular concern outlined by investors we interviewed include:

- ▶ Defining and predicting potency of the active ingredient
- ▶ Standardization of practices across multiple clinical sites
- ▶ Developing clinical infrastructure

First, defining minimum potency standards is needed. Currently, a dose is defined as a number of cells administered. But a cell therapy dose consists of a heterogeneous mix of cells. As we discussed, selection of T-cell populations and efficient CAR transduction to these cells can significantly affect the potency of the cell product. However, as we are still defining important characterizations of these cellular therapeutics, qualitative standards defining requirements to obtain minimum clinical efficacy have not yet been established. Until then, demonstrating clinical equivalency when each batch is patient specific ($n = 1$) remains a significant challenge to success in clinical trials, regulatory approval and production on a commercial scale.

Second, clinical practices must also be standardized, such as the establishment of preparative regimens and treatments for adverse events. The importance of preparative regimens has recently been underscored by the deaths in Juno's clinical trial for ALL using JCAR015. A balance must be struck in providing the correct lympho-depleted environment without causing adverse effects.

Lastly, the clinical infrastructure is needed to scale from single sites, where the majority of clinical trials are currently conducted, to the multiple sites necessary for successful commercialization of these therapies. Clinical trials now are typically performed at a single center with deep medical expertise. We need the clinical infrastructure, expertise and standardization to scale from one clinical site to many. Otherwise these therapies will not be successful in reaching larger patient populations and markets.

Commercial challenges

To gain perspective on the biggest barriers to the expansion of commercial opportunities for CAR-T therapies, we interviewed VC firms who actively invest in this space. While multiple areas for improvement were discussed, three key areas of focus emerged including:

- ▶ Availability of Good Manufacturing Practice (GMP) facilities
- ▶ Reduction in the cost of goods sold
- ▶ Application to bigger market opportunities

"Producing autologous CAR-T cells requires at least two unique types of facilities/processes. First, a virus manufacturer to produce retro- or lentivirus to modify the cells, and then also a cell manufacturing facility to process the patients' cells, transduce them with the virus, and then expand, characterize and formulate them. Currently both types of facilities have long wait lists and additional ramp up is needed to scale supply." (Michael Gladstone, Atlas Ventures). This 18-month waitlist exists both at commercial manufacturing sites as well as academic and institutional manufacturing

facilities [Nelsen Biomedical Interviews]. This is an obstacle for those working to commercialize CAR-T therapy, and for investigators working on new approaches.

Cost of goods is an additional barrier that has been widely discussed [51–55]. The first FDA-approved dendritic cell (DC) therapy, Provenge®, was developed by Dendreon. DCs stimulate antigen-specific cytolytic and helper T-cell responses, which lead to the formation of immunological memory. This capability has been used as a strategy to develop autologous cancer vaccines. Despite the efficacy of Provenge, the commercial challenges posed by manufacturing resulted in Dendreon filing for bankruptcy. How then, can companies attempting to commercialize autologous CAR-T therapies avoid the same fate?

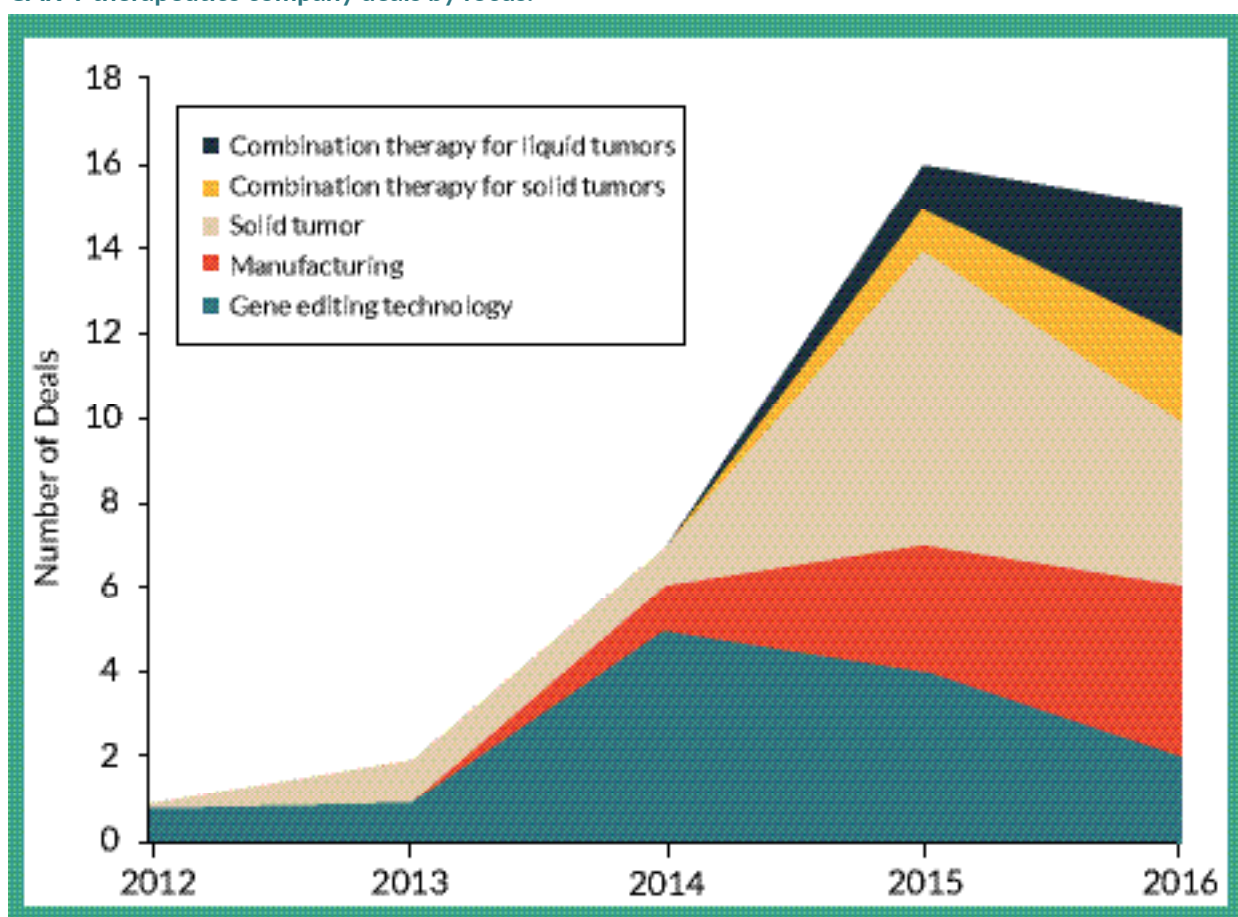
Fortunately, the industry has been working on developing more efficient and cost-effective solutions for manufacturing therapies where small-scale is full-scale. Shifts to disposable, small-footprint solutions as well as semi- or fully-automated, entirely enclosed manufacturing systems can reduce costs due to personnel, clean rooms and consumables. Notably, some of these automated, enclosed 'plug-and-play' systems in development may ultimately be able to manufacture cells from up to 30 donors in parallel. Putting this into perspective, one CEO of a cell therapy Contract Manufacturing Organization (CMO) noted that "many of our clients' clinical programs are such that if they're successful they will need to increase their current use from one to ten clean rooms a month, to 100 clean rooms a month. That's simply not

feasible. The concept of having a fully enclosed system that could process in parallel 30 patients at a time is very appealing.” (M Bamforth, Brammer Bio). At least ten closed manufacturing options are in development or on the market, with more improvements on the way [56]. The need to incorporate effective manufacturing has clearly been recognized by companies in the field, with increasing numbers

of partnerships being executed to focus on manufacturing (Figure 4). In fact, despite the stage of development, companies have begun to invest in automated manufacturing solutions. For example, with CAR-T therapy in just their pre-clinical pipeline, GlaxoSmithKline has recently entered into an agreement with Miltenyi Biotec to automate manufacturing and overcome scale-up constraints. In addition,

► FIGURE 4

CAR-T therapeutics company deals by focus.



As gene editing is integral to the CAR-T platform, companies began to form strategic partnerships to access or to license gene editing technology in 2012. The number of gene editing deals peaked in 2014 and has since been on the decline. Access to GMP facilities and cost of goods sold are significant commercial challenges to the manufacture of CAR-T therapies. Beginning in 2014, companies developing CAR-T therapies have formed strategic partnerships with GMP manufacturing facilities and companies providing efficient and cost-effective manufacturing solutions. Since then the number of manufacturing deals has been steadily increasing. Many believe that therapeutic success of CAR-T in treating solid tumors is critical to commercial success. In 2013, companies developing CAR-T therapies began forming partnerships and licensing agreements for acquisitions of technologies to develop CARs to treat solid tumors. The majority of these deals were made in 2015. One strategy to improve efficacy of CAR-T therapies is to combine them with a small molecule such as an antibody, checkpoint inhibitor or other signaling molecule. Since 2015 there has been an increase in the number of deals made for developing combination therapies for treating both solid and liquid tumors. See Supplementary Table 1 for additional details.

Sources: Company press releases and Nelsen Biomedical Analysis.

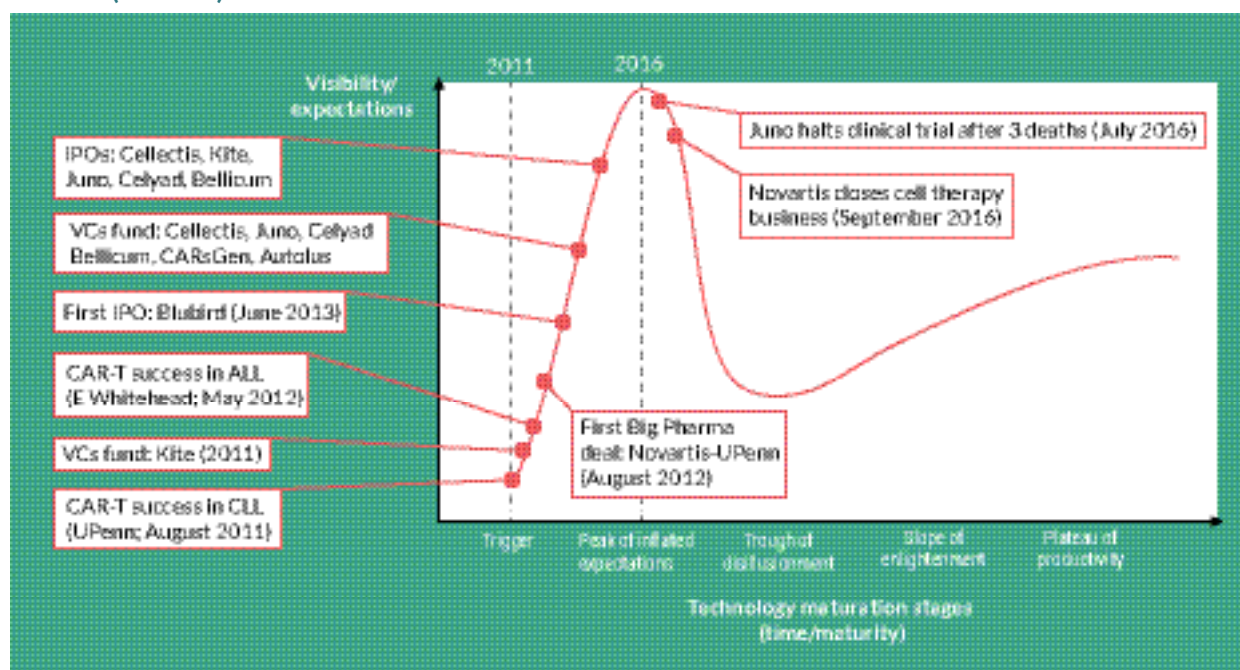
the later clinical stage CAR-T company, Juno, has acquired Cell Stage Therapeutics, in part for their next-generation automated manufacturing technologies in addition to their cell selection and activation capabilities (**Supplementary Table 1**, nelsenbiomedical.com/cartdeals).

Of course, to avoid this issue of scale out, one solution is to develop allogeneic approaches, which are favored by Big Pharma (**Table 3**). There were encouraging clinical results from the first patient treated with Cellectis 'off-the-shelf' CAR-Ts [58–60]. It's also worth noting that recently both Juno and Kite have made investments in developing off-the-shelf allogeneic approaches (**Supplementary Table 1**, nelsenbiomedical.com/cartdeals).

Application to bigger market opportunities is the third point cited by investors as a significant challenge for CAR-T therapies. According to an experienced investor in this field, "most believe growth in the industry will stem from successes in solid tumors and that if we can't figure solid out the CAR-T industry will stall". While we are likely to see registration of CAR-T products for blood cancers such as ALL and NHL in the next few years, CAR-T for solid tumors is a long way behind. A variety of approaches are underway in an attempt to translate the successes of CAR-T therapies in treating hematologic malignancies to solid tumors, as discussed above. But it is worth noting again that the industry and clinicians will always

► FIGURE 5

The rise (and fall?) of CAR-T.



Clinical successes using CAR-T therapies to treat patients with CLL in August 2011 and to treat Emily Whitehead with ALL in May 2012 triggered the excitement in CAR-T cellular therapeutics. Soon after came many firsts in the cell therapy industry. The first Big Pharma strategic partnership between Novartis and UPenn in 2012, followed by first VC funding to Kite in 2013 and the first IPOs of Bluebird Bio in 2013. Through 2016, other CAR-T therapeutics companies have raised VC dollars and completed IPOs (**Figure 1 and Tables 1 & 2**). With the recent deaths of three ALL patients during Juno's Phase 2 CAR-T clinical trial and Novartis' announcement that it is closing down its cell and gene therapy unit, will the industry adjust expectations and enter 'the trough of disillusionment'?

ALL: Acute lymphoblastic leukemia, CLL: Chronic lymphocytic leukemia, IPO: Initial public offerings, VC: Venture capital.

Sources: [61–64].

adopt the simplest, safest solution that is effective. For solid tumors there are many approved therapeutics and more in development, from small molecules to monoclonal antibodies to combination therapies that provide some level of efficacy. A complicated, expensive cell therapeutic will only be adopted if it provides a significant improvement in clinical outcome compared to other options.

A quick look at recent deal types highlights the main challenges the industry is trying to address, from manufacturing, to easier CAR creation using gene editing, to improving efficacy with combination therapies (Figure 4). A more comprehensive view of the details of these recent deals can be found in [Supplementary Table 1](#) nelsenbiomedical.com/cartdeals.

MOVING THE NEEDLE FOR CELL THERAPY

The significant, startling successes of CAR-T therapies in patient populations with no therapeutic solution have provided the evidence needed for the cell therapy industry to gain interest from investors, the public markets and Big Pharma. However, the recent deaths in the Juno trial for ALL using JCAR015, and the exit of Novartis from the cell therapy industry, force us to revisit the practical and clinical realities of these types of therapies for broader market applications and adoption. Right now, everything about them is unwieldy, from collecting the initial donor material, to manufacturing individual batches of autologous cellular therapeutics, to developing pre-conditioning regimens.

Where are we now? The excitement in CAR-Ts has perhaps hit its peak until the additional technology, clinical and commercial infrastructure required matures (Figure 5). Fortunately, the past few years have seen increased activity and development of the supporting services and infrastructure needed for the entire cell therapy industry to move forward. Manufacturing solutions and services designed to reduce the cost of goods, clinical infrastructure and development of standard operating procedures to improve safety, efficacy and availability of treatment are just a few of the areas where investment and improvements are occurring. Simpler, cheaper methods for genetic modification for a variety of cells will also help to move the field forward. Comments Michael Gladstone of Atlas Ventures “If the [clinical and manufacturing] infrastructure can be built, it will lay the groundwork now for a better and more potent generation of therapies.” Beyond just advancing CAR-T therapeutics, “CAR-T therapy is also paving the way for development of other potentially effective cell therapy, including TILs, NK cells and $\gamma\delta$ T cells.” (Shelly Chu, Abingworth). The advancement of even a single CAR-T therapy for a small patient population all the way to FDA approval will set a precedent that clears the way for the proliferation, adoption and commercialization of additional cell therapies.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

The authors have no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies,

honoraria, stock options or ownership, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

This article was written with hope and anticipation for this future of curative solutions, and in memory of all those who have struggled waiting for this new age of cancer medicine, particularly William and Gloria Nelsen.

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AFFILIATIONS

Michelle J Smith*, Brittni M Peterson* & Barbara A Nelsen[§]

Nelsen Biomedical, Minneapolis, MN, USA

[§]Author for correspondence

barbara@nelsenbiomedical.com

*Contributed equally to the work.