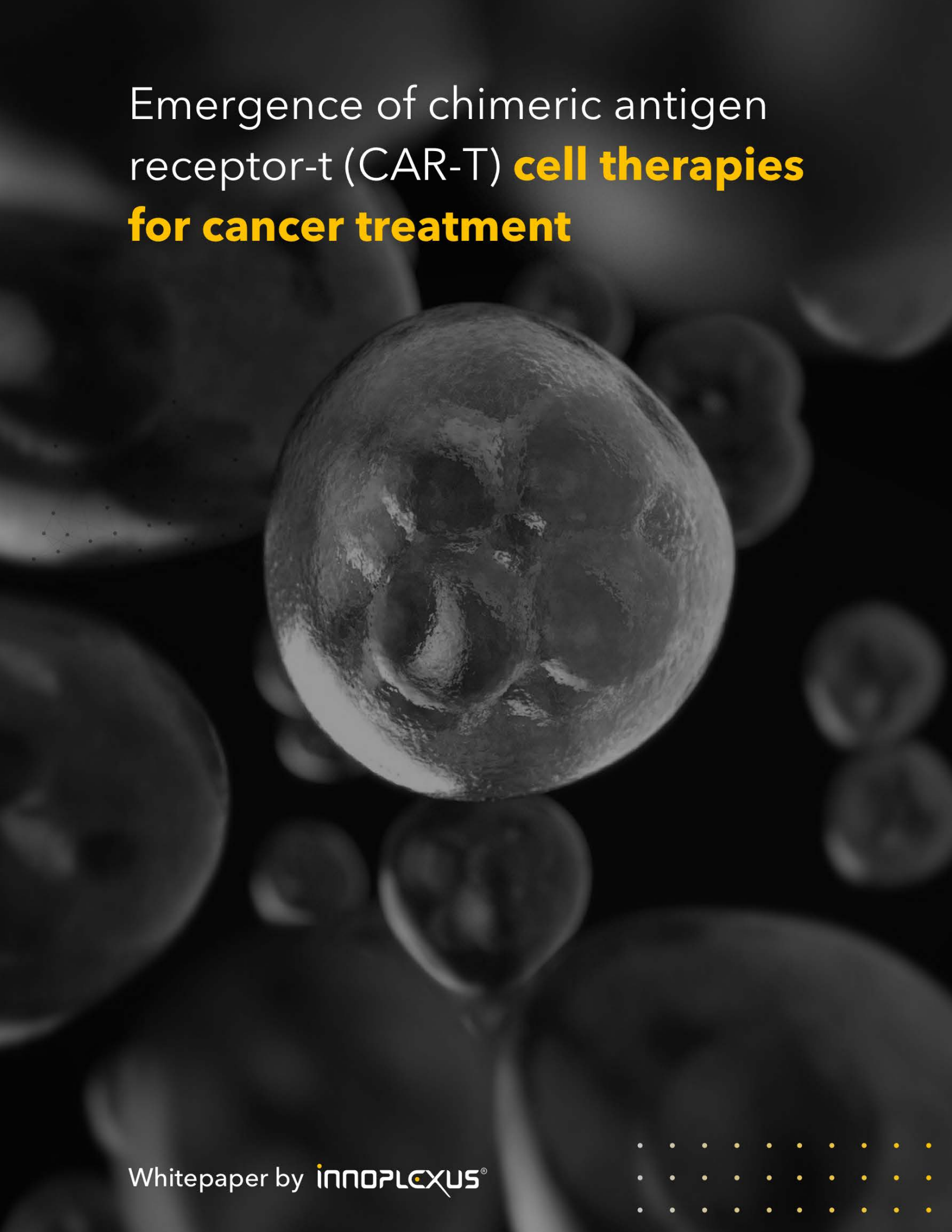
Emergence of chimeric antigen receptor-t (CAR-T) **cell therapies for cancer treatment**



Whitepaper by INNOPLEXUS®

1. About adoptive cell therapies and CAR-T cell therapy

1.1 Emerging interest in adoptive cell therapies (especially CAR-T cells)

Adoptive cell therapies or cellular immunotherapies provide treatment to patients, especially cancer patients, by using cells of their immune systems[1]. Immune cells isolated from the patient's body are extracted and expanded in number and/or genetically modified to enhance their therapeutic efficacy. These offer the potential for better and more personalised cancer treatments. The significance of approvals of these types of treatments was noted by the former FDA Commissioner, Scott Gottlieb, who in his October 2017 press release stated that they represent milestones "in the development of a whole new scientific paradigm"[2]. With the approval of the first cell-based gene therapy for adult patients with multiple myeloma in March 2021, FDA CBER Director Peter Marks stated "The FDA remains committed to advancing novel treatment options for areas of unmet patient need"[3]. With the potential to disrupt cancer care, these applications are currently limited to treating patients with select blood cancers in the relapsed or refractory stage.

Analysts project that the global CAR-T cell therapy market is expected to grow from $734 million in 2019 to $2,250 million in 2023 at an annual rate of 32.3%[4]. This projected growth may be attributed in part to a rise in the number of blood cancer cases: American Cancer Society estimates that new cases of leukemia (Figure 1), lymphoma and myeloma account for ~9.9% of the estimated 1,806,590 new cancer cases diagnosed in the US in 2020[5]. Additionally, there is also a growing awareness of these kinds of therapies. More support from the FDA in terms of guidance documents to conduct CAR-T therapy-related trials would serve as further encouragement.

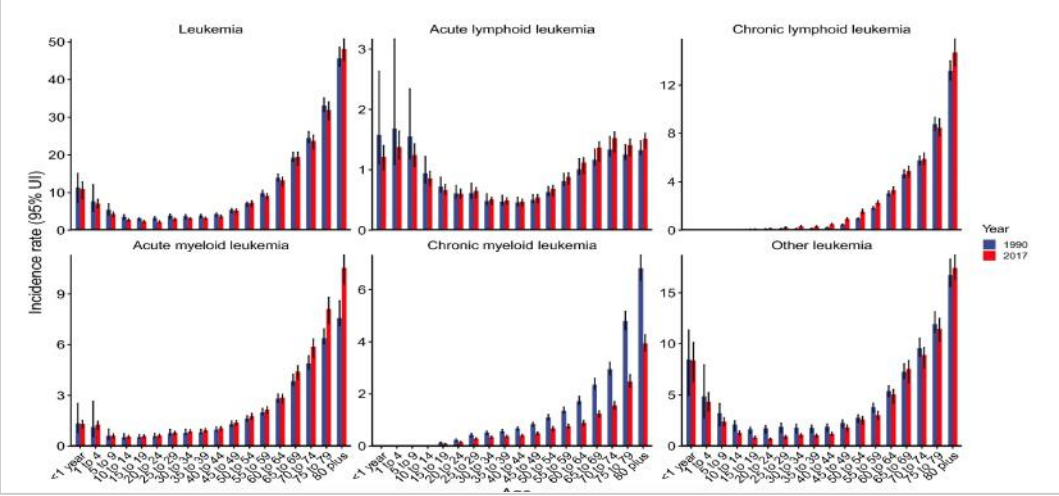


Figure 1: Incidence rate of leukemia by type and year among people of different ages[6]

1.2 Different type of adoptive cell therapies and their mechanism of action

Cellular immunotherapies leverage the potential of the body's natural immune response - especially killer T-cells and their ability to recognize antigens presented on the surface of cancer cells. Several mechanisms by which these therapies can be deployed are[1]:

1. Chimeric Antigen Receptor (CAR) T Cell Therapy
2. Engineered T Cell Receptor (TCR) Therapy
3. Natural Killer (NK) Cell Therapy

1.2.1 Chimeric Antigen Receptor (CAR) T Cell Therapy

CAR-T cells are genetically modified T-lymphocytes expressing chimeric antigen receptors. A CAR consists of an antibody-derived single-chain variable fragment (scFv) linked via a spacer and transmembrane domain to intracellular signaling molecules. Using this adoptive cell transfer therapy, a patient's own or a donor's blood cells are genetically modified and used to kill specific cancer cells[7]. CAR-T cells recognize and kill tumor cells independently of major histocompatibility complex (MHC) molecules by enabling T cells to bind target cell surface antigens through the scFv recognition domain. They have been approved to treat different types of lymphomas and are also being studied for the treatment of other types of cancer[8].

CAR-T cell development process (Figure 2) consists of three main steps[9]:
a. Isolation and enrichment of T cells by leukapheresis
b. CAR-T cell preparation, including T cell activation and expansion, gene transfer with a CAR vector while using viral or non-viral vector systems, followed by ex vivo CAR-T cell expansion
c. The final cell product is subjected to end-of-process formulation and cryopreservation
Before administration of the finally approved CART cell product, cancer patients usually receive a lympho-depleting treatment

After leukapheresis, before the modified CAR-T cells are transfused, most patients undergo a lymphodepleting chemotherapy (conditioning regimen) that helps in creating a favourable environment for immunotherapy, along with persistence and clinical activity of CAR-T cells.

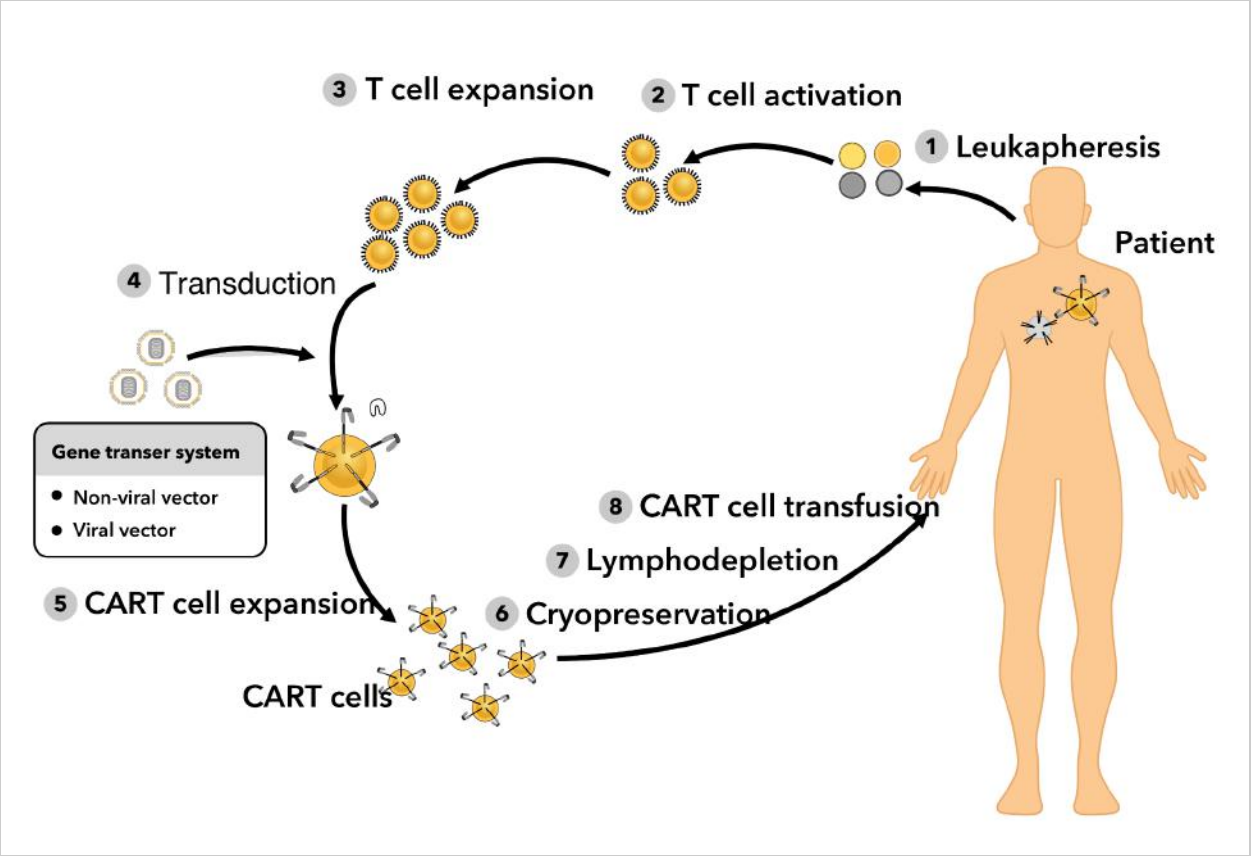


Figure 2: Preparation and administration of CAR-T cells[9]

1.2.2 Engineered T Cell Receptor (TCR) Therapy

This type of immunotherapy primarily focuses on the T-cell receptors (TCR) in which a patient's own T-lymphocytes are modified *ex vivo* before being injected back into their body. The major difference with CAR-T cells lies in the ability of TCR therapy to use TCR- peptide/MHC interaction to eradicate tumor cells, especially solid tumors. Intracellular tumor-related antigen fragments are presented as peptides by the MHC on the cell surface, which then interacts with the TCR on antigen-specific T cells to stimulate an anti-tumor response. This therapy thus has a wide range of targets - any antigen that can be presented by a MHC molecule can be recognized by the TCR-T cells even if they are internal molecules of cancer cells or formed after mutation in a tumor cell[10].

1.2.3 Natural Killer (NK) Cell Therapy

To overcome safety-related and non-compatibility issues of CAR-T cell therapy, CAR-NK therapies have also come into focus as alternative CAR vehicles, especially in allogeneic settings[11]. NK cells can kill transformed cells without prior antigen priming or expression of the target cell's MHC molecules. Based on whether the inhibitory or activator receptors are expressed by healthy or "stressed" cells respectively, NK cells can selectively kill tumor ("stressed") cells, thereby reducing the on-target/off-tumor toxicity to normal tissues. Adverse events associated with CAR-T cell therapy, eg. cytokine release syndrome (CRS)

and neurotoxicity are less likely to occur with CAR-NK cells. These cells are also less susceptible to host-versus-graft rejection in allogeneic settings, making them a safe choice as a third-party, off-the-shelf cellular therapy option[12].

1.3 Evolution of CAR-T cell therapy over the years

Immunotherapies to target cancer cells have been the focus of translational research for over two decades. Several methods to activate the immune system have been used to target and kill cancer cells. Adoptive cellular immunotherapy approaches identified ways to re-engineer antitumor immunity with some initial success and FDA approvals[13,14]. Some major milestones in the development are illustrated below (Figure 3).

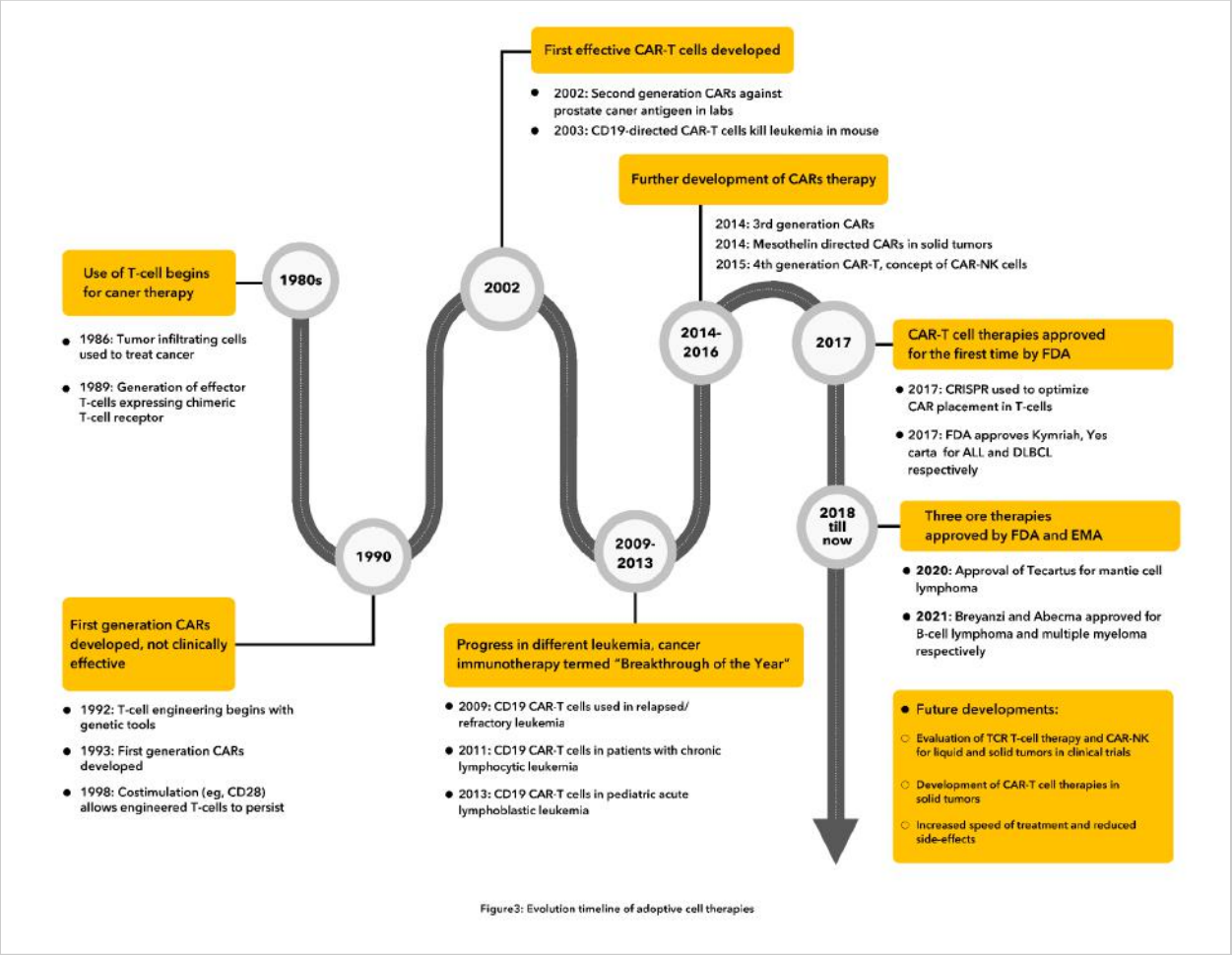


Figure 3: Evolution timeline of adoptive cell therapies

1.3.1 Structural evolution of different chimeric antigen receptor generations

CAR-T cells have extracellular, transmembrane and intracellular domains[15] (Figure 4a):

- The extracellular domain has a single-chain variable fragment (scFv) formed by the variable portions of heavy and light chains of an immunoglobulin fused through a flexible linker domain for the recognition of tumor-associated antigens with specificity and affinity
- The transmembrane domain consists of a hydrophobic alpha helix that spans the membrane
- The intracellular domain consists of immunoreceptor tyrosine-based activation motif (ITAM) of the T cell receptor complex CD3ζ chain, which activates the costimulatory signal

CAR-T cells are divided into four generations (Figure 4b) based on their signalling domains[15]:

a. **First generation**: These cells could induce T cell activation only by the primary signal via the CD3 ζ- chain or FcεRIγ signaling domain and without a costimulatory domain. However, it was necessary to administer exogenous interleukin-2 (IL-2) with these CAR-T cells as they could not produce enough IL-2 in order to achieve tumor cell destruction by promoting T-cell cytotoxic activity.
b. **Second generation**: Second generation CARs consist of three different receptor types including the T-cell antigen receptors, cytokine receptors and co-stimulatory receptors eg. CD28, 4-1BB and CD134 (OX40) engineered to the signal transduction region. This enhances proliferation, cytotoxicity, sustained response of the CAR-T cells and also prolongs their life *in vivo*.
c. **Third generation**: The third-generation CARs are generated by combining multiple signaling domains, such as CD3ζ-CD28-OX40 or CD3ζ-CD28-41BB, this augmented their potency with stronger cytokine production and killing ability. Results from preclinical studies have shown that these CAR-T cells (with 4-1BB and CD28 signalling domains) have superior activation and proliferation capacity compared with 2nd-G CAR-T cells carrying CD28 domain. However, addition of a costimulation domain may produce severe side effects and accelerate the aging of CAR-T cells.
d. **Fourth generation**: In the fourth-generation CARs, IL-12 is added to the base of second-generation constructs, and is called “T cell redirected for universal cytokine-mediated killing (TRUCKs)”. TRUCKs augment T-cell activation and activate and attract innate immune cells to eliminate antigen-negative cancer cells in the targeted lesion. TRUCK T cells are envisioned to be applied in fields beyond cancer therapy including the therapy of virus infections, auto-immune diseases or metabolic disorders.

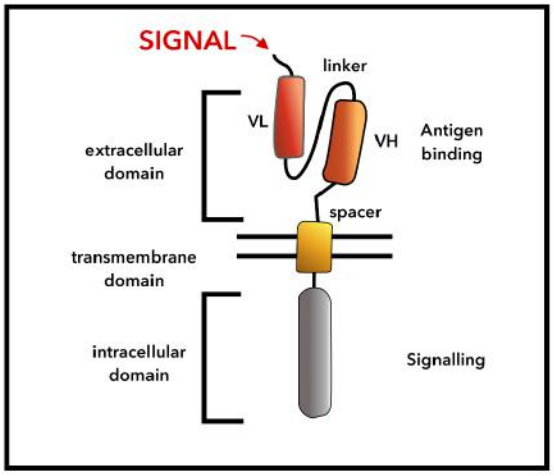


Figure 4a: Structure of a chimeric antigen receptor

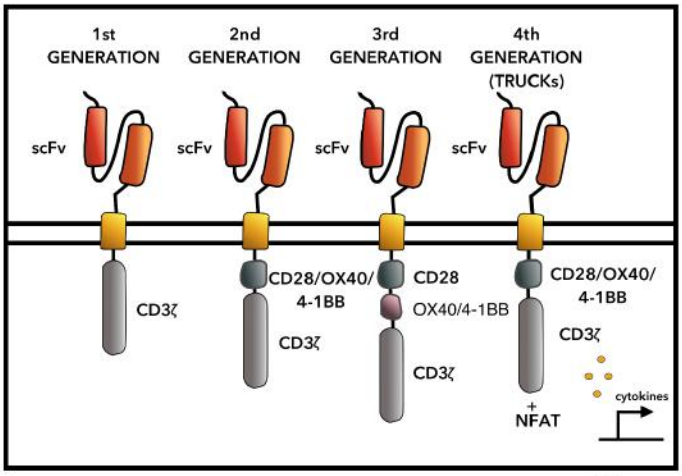


Figure 4b: Chimeric antigen receptor generations[16]

1.4 Combination of Immune Checkpoint inhibitors with CAR-T Cells

CAR-T cells provide a highly specific antitumor immune response which can be further enhanced by the addition of checkpoint blockers[17]. This combination can counteract the immune inhibitory environment that reduces optimal CAR-T cell efficacy. Immune system checkpoints such as programmed cell death protein 1 (PD-1, PD-L1) and cytotoxic T-lymphocyte-associated protein-4 (CTLA-4), play important role in balancing T-cell activation and inhibition, as well as preventing autoimmunity.

There are two strategies of combination - Cell Intrinsic and Cell Extrinsic methods. Cell-intrinsic PD-1 Checkpoint Blockade (CPB) includes genetic engineering to express nucleic acids or proteins that interfere with PD-1/PD-L1 signaling, while cell-extrinsic CPB relies on PD-1 receptor/ligand blocking antibodies.

Table 1 discusses the limitations and benefits of both methods of combination preparations:

Parameter	Cell-intrinsic approach	Cell-extrinsic approach
Potential dosage	Single	Multiple doses of Checkpoint blockade
Targeted therapy	Yes	No
Potential toxicity	Localized to tumor	Systemic
Limits to tumor penetration	Unlikely	Limits of antibody penetration

Table 1: Characteristics of methods used for combination of CPB with CAR-T cells

Several clinical trials are ongoing for combination therapy with CPB and CAR-T cells for different conditions that include B cell lymphoma, Chronic lymphocytic leukemia, Acute lymphocytic leukemia, Neuroblastoma, Non-Hodgkin lymphoma, etc.

1.5 Persisting challenges in CAR-T cell therapy clinical development

CAR-T cell therapy has achieved spectacular results, but still there are few challenges which include severe and common adverse reactions as well as high costs associated with it[18].

a. Toxicity

Major obstacles in using CAR-T cell therapy are the toxicities associated with it, primarily cytokine release syndrome (CRS) and neurologic toxicity. Factors that may influence toxicity include levels of certain cytokines, peak blood CAR T-cell levels, CAR T-cell dose, endothelial activation, also, CAR design. This has been discussed in detail in Section 1.6.

b. CAR Costs

Even though the clinical applications of approved CAR T-cell therapy are still in their earliest stages, the cost of treatment is enormous. In the USA, treatment with Yescarta® costs USD 373,000 (EUR 316,000), whereas Kymriah® therapy comes up to USD 475,000 per patient (EUR 400,000). This is because of the complexity of CAR T-cell therapy, which requires personalised manufacturing for each patient and sample transportation between specialized centers. The prohibitive cost makes these therapies inaccessible for most patients and has led to multiple restrictions in European countries[18].

c. Antigen selection

Identifying ideal target antigens other than CD19 (optimal target with a high and universal expression on tumor cells, no or low expression on normal cells) is very challenging for solid tumors[20]. Most of the CARs to date have targeted cell surface molecules without these optimal characteristics. New preclinical targets are being identified using advances in RNA sequencing, microarray analysis, and proteomics, such as the recent discovery of glypican2 (GPC2) in neuroblastoma.

d. Trafficking CAR T Cells to the Tumor Site

Chemokine receptors can help systemically administered CARs to reside at the tumor site. This strategy is challenging because multiple types of chemokines are secreted by the tumors[20]. One preclinical model showed that chemokine receptor type 2b (CCR2b) expression on GD2-CAR T cells improved residing in neuroblastoma tumor cells and pleural mesotheliomas. Another approach to overcome this challenge is to deliver CAR-T cells directly into the tumor or in close proximity.

e. Hostile Tumor Microenvironment (TME)

The tumor mass consists not only of a heterogeneous population of cancer cells but also a variety of secreted factors, extracellular matrix proteins, resident and infiltrating host cells, which is collectively known as the tumor microenvironment[21]. Among others, a hostile TME may be caused by:

- Presence of inhibitory cells such as T-regulatory cells
- Tumor-associated macrophages, or myeloid-derived suppressor cells (MSDCs)
- Chronic T cell receptor signaling that leads to T cell exhaustion

Sometimes, TME may be immunosuppressive to CAR-T cell therapies. Also, the stroma and the extracellular matrix can inhibit effective migration to cancer cells. To overcome these challenges, scientists are using various different strategies eg. one of these strategies is engineering of CAR-T cells to produce heparanase which demonstrates improved penetration of CAR cells within tumors by degrading the extracellular matrix. Research is also being carried out on cancer-associated fibroblasts that play an important role in extracellular matrix remodeling[20].

f. T cell Exhaustion:

T-cell exhaustion is a progressive loss of effector function due to characteristics of chronic infections, prolonged antigen stimulation, and cancer. In addition to continuous antigen stimulation, cytokines and antigen presenting cells which exist in the microenvironment can also result in T-cell exhaustion[22]. Due to this, tumor cells can undergo a dynamic metabolic reprogramming (utilizing metabolic byproducts as alternative fuel sources) which confers cell plasticity and survival in such harsh conditions while concomitantly further depriving T-cells of nutrients essential for their survival and function[23].

1.6 Adverse events associated with CAR-T cell therapies

The adverse events associated with CAR-T cell therapy are different from traditional chemotherapies, monoclonal antibodies, and small molecule therapies. CAR-T cells may cross-react with a protein on normal tissues that is differentially expressed on tumor cells causing damage to normal tissues. The most common toxicities observed after CAR T-cell therapy are Cytokine Release Syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). ICANS are observed in CAR-T cells that target CD19 and the risk of this association may be mitigated by detecting plasma biomarkers present during apheresis[19]. Other adverse reactions include "on-target, off-tumor" recognition and anaphylaxis including adverse events that occur after CAR-T cell infusion[24].

The most widely occurring adverse events with CAR-T cell therapies are[25]:

a. Cytokine release syndrome (CRS)

Cytokine release syndrome has been observed with CAR-T cell therapy and also other T-cell receptor gene therapies and bispecific T-cell engaging antibodies. Commonly observed symptoms of CRS are high fevers, hypotension, hypoxia, and other organ toxicities.

After binding CAR-T cells to their target antigen, they proliferate, produce cytokines, and increase the expression of cytotoxic molecules that mediate the destruction of cancer cells. These destroyed cells and other immune effector cells release an excess of cytokines into circulation, which is called a cytokine storm. Elevated levels of interferon-γ, granulocyte macrophage colony-stimulating factor, interleukin (IL)-10, and IL-6 have been observed following CAR-T cell infusions. This cytokine storm results in vasodilation, decreased cardiac output, and intravascular volume depletion[26]. Tisagenlecleucel showed 77%[27] and 57%[28] incidence of CRS in patients with Acute lymphocytic leukemia (ALL) and Non-Hodgkin lymphoma (NHL), respectively. Tocilizumab effectively lowers CRS-related toxicities following CAR-T cell infusions.

b. Central Nervous System (CNS) toxicity including ICANS

Neurologic toxicities range from mild symptoms including headache, dizziness, confusion and trouble sleeping to more severe ones such as aphasia, ataxia, delirium, seizures, cerebral edema, and coma, known as Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). The onset of CNS toxicity occurs concurrently with CRS in most cases and in less than 10% of patients, it is observed without cytokine release.

Although the neurotoxicity due to CAR-T cells is not fully understood, recent studies showed that dysfunction of the blood brain barrier, formed by capillary endothelial cells surrounded by extracellular

matrix, pericytes, microglia and astrocytes could be the main factor. In severe forms of CAR-T cell neurotoxicity, molecules that induces expansion and activation of CAR-T cells (IFN-γ, IL-10, G-CSF, GM-CSF, IL-8, MCP-1) and neurotoxic substances such as glutamate and quinolinic acid, have been found to be elevated[24]. After treatment with Tisagenlecleucel, around 40% neurological toxicities were reported for ALL and NHL occurred within the first 8 weeks.

c. Other toxicities associated with CAR-T cells

- Infusion reactions: Commonly observed events are upper digestive symptoms eg. nausea related to the DMSO cryoprotectant and vomiting and hypotension due to diphenhydramine premedication.
- Cytopenias: Neutropenia is the most common cytopenia observed in patients treated with CAR-T cells. Axicabtagene ciloleucel and tisagenlecleucel treatments have grade ≥3 cytopenias along with severe thrombocytopenia present for several weeks following the CAR-T cells infusion.
- Cardiac toxicity: The incidence of cardiac events such as sinus tachycardia, heart failure, arrhythmias, hypotension, shock, or cardiac arrest have been observed with CAR-T cell infusion. These AEs were observed with Tisagenlecleucel or axicabtagene ciloleucel ranging from 29% to 39% patients.
- Hypogammaglobulinemia: It is a delayed side effect in patients with CLL who received CD19-directed CAR-T cell therapy. Hypogammaglobulinemia is mainly associated with a complete response with decrease in IgG levels within 1 to 3 months after CAR-T infusion and can remain low up to 4 years.

1.7 FDA approved CAR-T cell therapies

Five CAR-T cell therapies have been approved by FDA till date for the treatment of advanced B-cell lymphomas and recently for multiple myeloma[29] (Table 2). These agents use different types of genetic engineering methods to transform the patient's T cell to CAR-T cells. All these CAR-T cell therapies, except Abecma, bind to the protein antigen CD19 (found on the surface of B-cells). Abecma binds to the target antigen protein BCMA, which is found almost exclusively on the surfaces of malignant plasma cells (not expressed in their normal counterparts or other cell types)[30], making it a compelling target for treating multiple myeloma patients.

Drug	Brand Name	Condition approved for	Approval Year
Idecabtagene vicleucel	Abecma	Relapsed or refractory multiple myeloma after four or more prior lines of therapy	2021
Lisocabtagene maraleucel	Breyanzi	Relapsed or refractory large B-cell lymphoma after two or more lines of systemic therapy	2021
Brexucabtagene autoleucel	Tecartus	Relapsed or refractory mantle cell lymphoma	2020
Tisagenlecleucel	Kymriah	-Relapsed or refractory diffuse large B-cell lymphoma -Relapsed or refractory ALL (young adults upto age 25)	2017
Axicabtagene ciloleucel	Yescarta	Certain types of B-cell lymphoma (DLBCL, Primary mediastinal B-cell lymphoma, High grade B-cell lymphoma, DLBCL that results from follicular lymphoma, Follicular lymphoma)	2017

Table 2: Approved CAR-T cell therapies

1.8 Other T-cell based immunotherapies

Apart from CAR-T cell therapy, other categories of T-cell-based immunotherapies that are in clinical use include immune checkpoint inhibitors and bispecific antibodies (eg. BiTEs or bispecific T cell engagers). A number of immune checkpoint inhibitors have been approved for multiple malignancies but proved to be most effective for melanoma. Immune checkpoint inhibitors depend primarily on MHC mediated response of T cells to a variety of tumor associated antigens (TAAs). But, CAR-T cells and bispecific antibodies can kill cancer cells in MHC independent manner by redirecting cytotoxic T cells to predefined targets on cancer cells. BiTEs have two components (scFVs) - one of which binds to a selected TAA and the other directed to CD3 on T-cells. The MHC-independent targeting of TAAs results in cytotoxic release of perforin and granzyme B from endogenous T cells. FDA has approved the first-in-class BiTE, Blinatumomab, for adult and paediatric patients with R/R Ph−B-ALL, and for the treatment of MRD in adults with B-ALL who are in haematological complete remission. Because of their effectiveness in hematological malignancies, researchers are also trying to test their efficacy in solid tumors. Other similar technologies which are being developed to harness anti-cancer immune function, reduced immune-related adverse events are: bifunctional checkpoint-inhibitory T cell engagers (CiTEs), simultaneous multiple interaction T cell engagers (SMITEs), trispecific killer engagers (TriKEs) and BiTE-expressing chimeric antigen receptor(CAR) T cells (CART.BiTE cells)[31].

2. Market size and opportunity

With gradual advances in gene therapeutics, market size and investors' interest in companies that are dealing in CAR-T cell therapeutics has increased significantly.

2.1 Market size by year

Market for CAR-T cell therapy products is valued at US$ 1,081 mn in 2020 and has grown at a CAGR of more than 78% over 2018 to 2020 from a small base of US$ 340 mn in 2018 (Table 3). Going forward, the CAGR is likely to be over 38% to USD 10.4 bn[32]. Though, recent approvals to BMS for two CAR-T cell therapies may push the growth estimate forward.

Company	Drug	Approval Year	CAGR 2018-20 (%)	2020 (US$ mn)	2019 (US$ mn)	2018 (US$ mn)
BMS	Abecma	2021	NA	NA	NA	NA
BMS	Breyanzi	2021	NA	NA	NA	NA
Novartis	Kymriah	2017	149.7	474	278	76
Gilead	Yescarta	2017	46.0	563	456	264
Gilead	Tecartus	2020	NA	44	NA	NA
	Total		**78.3**	**1081**	**734**	**340**

Table 3: Market size for approved CAR-T cell therapies

Among 5 approved drugs, Gilead's Yescarta has logged the highest market share of 52% in 2020 while Novartis' Kymriah has emerged as the largest drug by growth by registering a growth CAGR of ~150% over 2018-20 to US$ 474 mn.

Approved in 2017, Kymriah generated highest growth of ~150% over 2018-20 at low base but Gilead's Yescarta, which got approval in 2017 generated more than 200 mn in revenues in first year of launch and grew at 46% over 2018-20 to 563 mn (52% market share in market for CAR-T cell therapies).

2.2 Rising interest in CAR-T cell therapy companies

With gradual successes in the CAR-T cell therapeutics, industry has witnessed consolidation as well as an increase in investor's interest which is evident from the increasing number of IPOs.

CAR-T cell therapy focused companies have witnessed a surge in M&A recently. There were ~13 deals during the last 4 years amounting to ~$15 billion as against just 4 deals prior to that period (Figure 5). Few notable deals being Gilead acquiring Kite Pharma for $12 billion in 2017, Catalent acquiring Paragon Bioservices for $1.2 billion in 2019 and Vertex Pharmaceuticals acquiring Exonics Therapeutics for $1 billion in 2019.

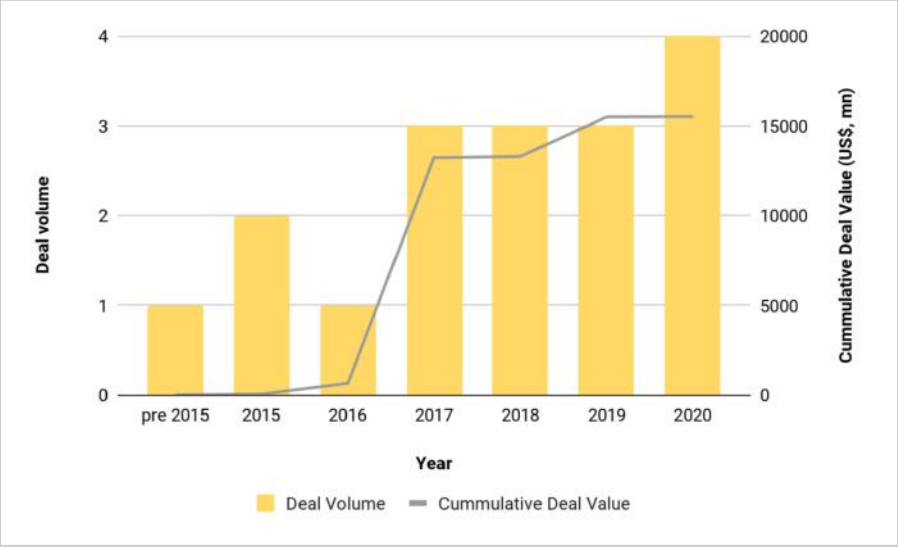


Figure 5: Year-wise and cumulative deal value (US$, Mn)

2.3 Fund raising via IPO route

Funds raised via the IPO route have increased consistently since 2018 till 2020. This trend continues in 2021 with the first quarter witnessing close to half of the funds raised in 2020 (Figure 6).

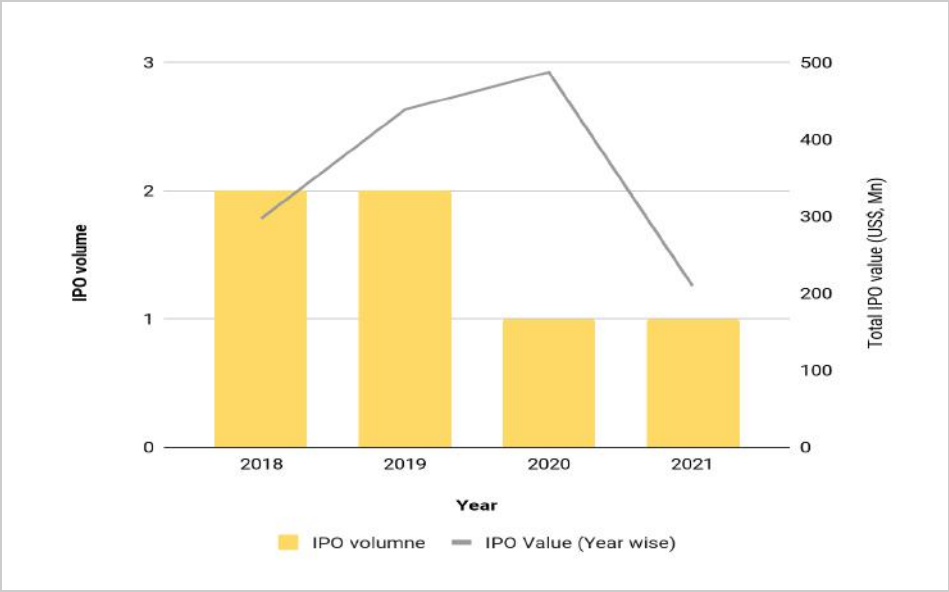


Figure 6: Year-wise IPO volume and value (US$, Mn)

2.4 CAR-T cell therapy company stocks were seen in sweet spot

Market cap of CAR-T cell therapy focussed companies has seen robust traction in the recent past. We have analysed returns on the market cap weighted portfolio of CAR-T cell therapy focussed companies having market cap less than US$ 20 billion, which significantly outperformed the healthcare indices. Below is the chart (Figure 7) showing the curve of market cap weighted returns from 1st January 2019 till 20th April, 2021.

Figure 7: Movement in stock percentage for midcap weighted portfolio

3. Descriptive statistics based on CAR-T cell therapy specific features

In this section, we provide a detailed report on clinical trials using CAR-T cell therapies by analysing therapy-related parameters such as target antigen, target organ, route of administration, CAR-T cell generations, etc. This trial landscape is also presented for trial parameters like conditions, sponsors, phases, start and completion years. CAR-T cell therapy trials are further analysed for safety and efficacy objectives.

3.1 Trends of trials

We have studied 888 trials of adoptive cell therapies including CAR-T cells, CAR-NK cells and TCR cells from the ClinicalTrials.gov registry, considering interventional trials with an adoptive cell therapy as an experimental drug (excluding Phase 4 trials). This corpus contains ongoing, completed and terminated trials.

3.1.1 Increase in phase-wise CAR-T cell therapies trials

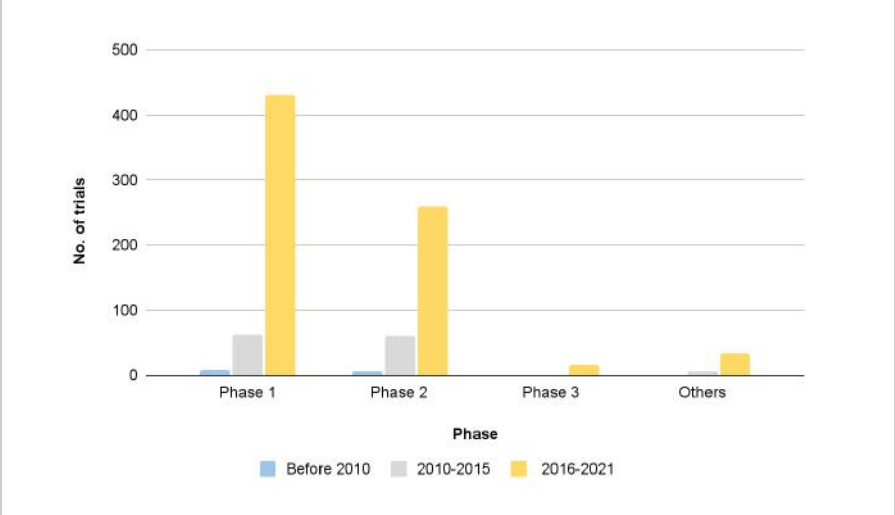


Figure 8: CAR-T cell therapy trial distribution by Start year and Phase

To observe the trends in initiation of CAR-T cell therapy trials, we segmented the trends of trials across different phases over the years. Of total 888 trials analysed, 402 are industry sponsored across different phases. Increasing interest in CAR-T cell therapy trials is observed in recent years as seen from peak in Phase 1 and Phase 2 trials (Figure 8). Number of trials initiated have increased by approximately 6 and 4 folds respectively in Phase 1 and 2. Though a lot of trials are initiated in Phase 1 and 2, most of them fail to reach Phase 3, which could be due to the safety issues associated with most CAR-T cell therapies.

3.1.2 Analysis by target organ by the start year of CAR-T cell therapy trials

Analyzing CAR-T cell therapy trials by target organs based on the conditions that they are studied in the respective clinical trials, we observed that CAR-T cell therapy is mainly studied in the lymphatic system and blood cells in oncological indications with leukemia/lymphoma being a major area of focus (Figure

9). Increasing trends of trials are also observed in organs that develops into solid tumors eg. brain, lung, liver.

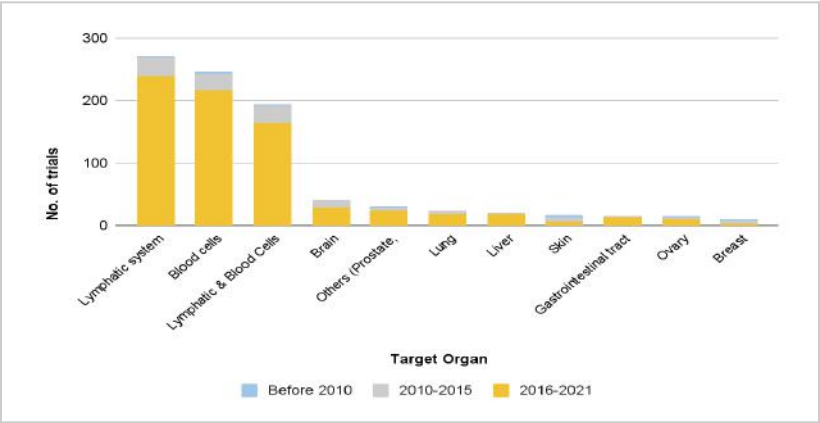


Figure 9: Distribution of CAR-T cell therapy trials by target organs and start year

3.1.3 Year-wise CAR-T cell generation analysis

Among the four generations of CAR-T cells (n= 586 trials) differentiated based on their signalling domains, the second generation CAR-T cells with CD28/ 4-1BB/ CD137 (OX40) as a signalling domain have been widely studied (82%) in clinical trials (Figure 10). Results from fourth generation CAR-T cells are highly anticipated as they hold the promise in solid tumors by modifying their tumor microenvironment through transduced cytokines.

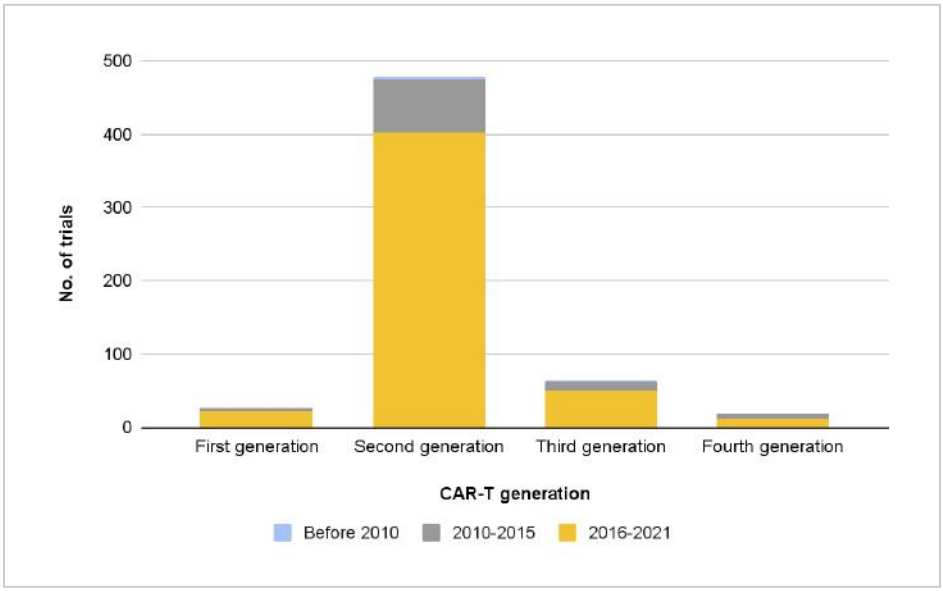


Figure 10 : Distribution by CAR-T cell generations and start year buckets

3.1.4 Signaling domain

Out of the 888 CAR-T cell therapy trials, 341 studies (38%) explored CAR-T cell therapies with CD3-zeta and 4-1BB signaling domains to activate T cells, 137 (15%) CD3-zeta and CD28, 125 (14%) other signaling

domains and 285 (33%) with unknown signaling domain. For most of the conditions, cell therapies have CD3-zeta signaling domain coupled to 4-1BB costimulatory domains except Hepatocellular Carcinoma studies where 60% have cell therapies with CD3-zeta and CD28 (Figure 11). Many trials are also being conducted using the combination of 4-1BB and CD28 costimulatory domains. Some studies report that CD28 costimulatory domains are associated with faster tumor elimination and lower activity at lower effector:target ratios compared to those expressing 4-1BB domains[33].

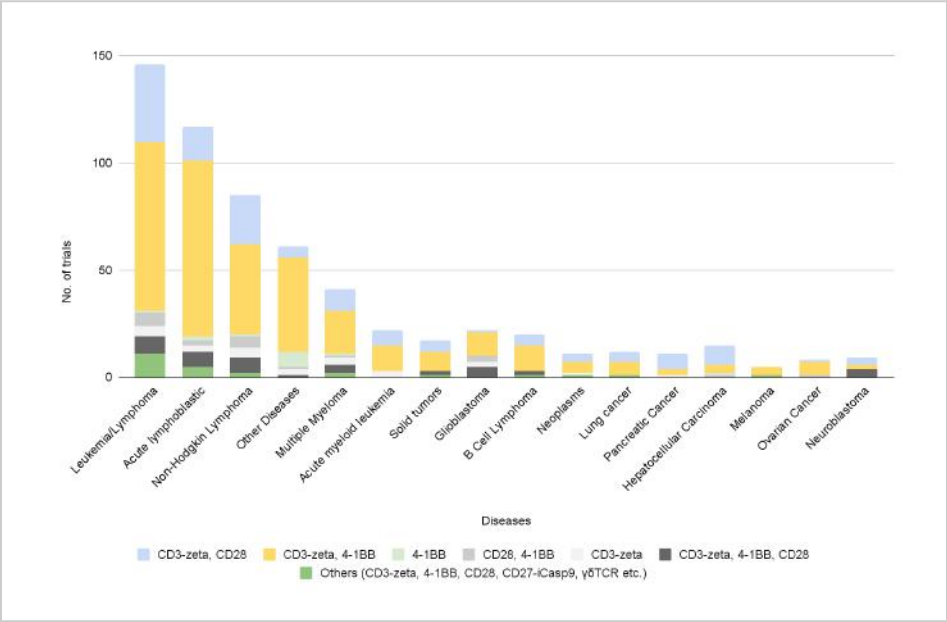


Figure 11 : Distribution of CAR-T cell therapy trials by signaling domain and condition

3.2 Top diseases for CAR-T cell therapies

We spotted the top diseases for which CAR-T cell therapy trials are being conducted across different phases. Most of the CAR-T cell therapy trials (873 trials, ~98%) are being conducted in the therapeutic area of Oncology (TA-Oncology) out of which 69% (617) trials are for hematological malignancies and the rest for solid tumors (256).

Top 15 diseases (Figure 12) covered 789 studies in TA-Oncology. Most of the CAR-T cell therapies are conducted for Leukemia/Lymphoma (195 trials, 22%) followed by Acute lymphoblastic leukemia (145 trials, 16%), Non-Hodgkin Lymphoma (99 trials, 11%), Multiple Myeloma (99 trials, 11%) and Acute myeloid leukemia (39 trials, 4%).

CAR-T cell therapies are also being studied in non-oncological diseases such as HIV Infections, CD19 Positive Systemic Lupus Erythematosus, Neuromyelitis Optica Spectrum Disorders, POMES Syndrome, etc.

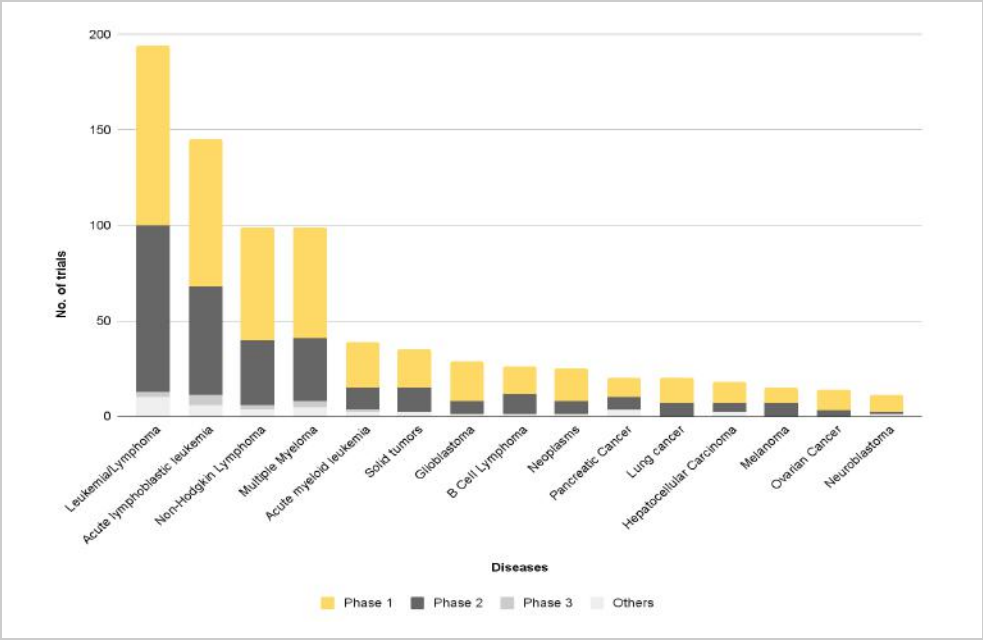


Figure 12 : Distribution of CAR-T cell therapy trials by top conditions and phase

3.2.1 Top diseases in Solid Tumors

Further in this subsection, we are analyzing the diseases in solid tumors where most studies using CAR-T cell therapy are done. There are 256 (28%) studies which are being conducted for solid tumors, 617 (69%) for hematological malignancies, and 15 (3%) for other conditions. Top diseases which are using CAR-T cell based therapies in solid tumors are Glioblastoma, Pancreatic Cancer, Lung cancer, Hepatocellular Carcinoma and Melanoma (Figure 13). Multiple solid tumors (35 trials) include various solid cancers such as Gastric Cancer, Breast Cancer, Neuroblastoma, Ovarian Cancer, etc, studied in a given single trial. One trial of pancreatic cancer has reached Phase 3 and is recruiting patients.

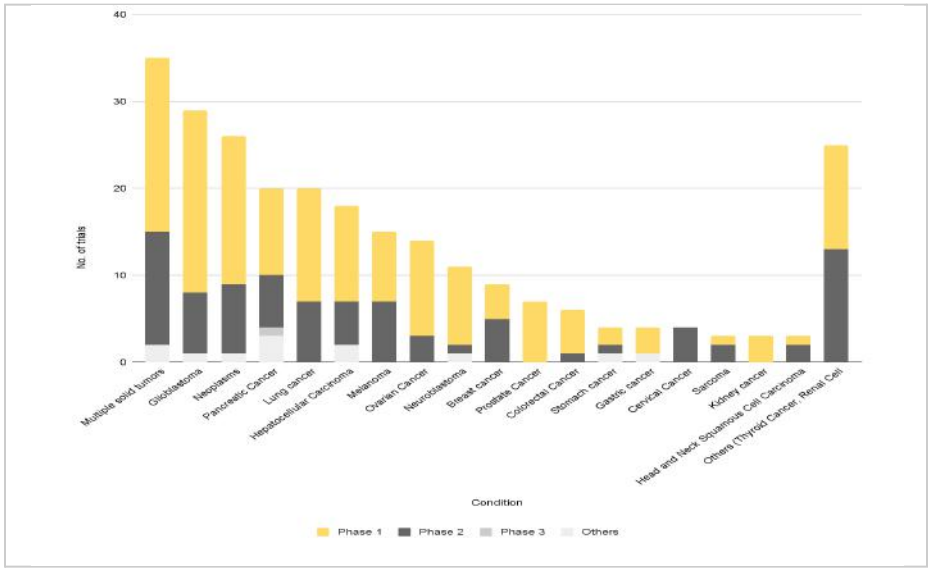


Figure 13 : Distribution of CAR-T cell therapy trials in Solid Tumors by conditions

3.3 Top Sponsors

In this subsection, we analysed the top ten sponsors contributing 244 CAR-T cell therapy trials (Figure 14). The sponsor studies showed National Cancer Institute (42 trials) with the highest interest in CAR-T cell therapy followed by University Of Pennsylvania (38 trials), Baylor College Of Medicine (25 trials), Novartis (22 trials) and Celgene (22 trials) in recent years. Novartis, Celgene and Gilead Sciences are the top industry sponsors which have advanced pipelines with trials in Phase 3 while National Cancer Institute and Shenzhen Geno Immune Med. Inst. covered most of the Phase 2 trials.

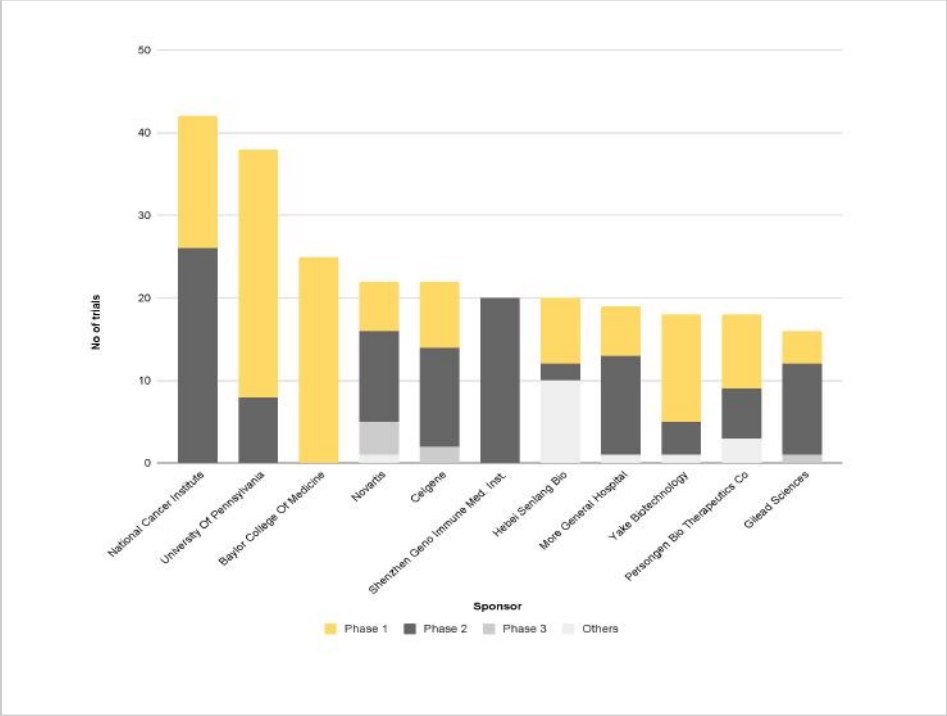


Figure 14 : Distribution of CAR-T cell therapy trials by top sponsors and phase

3.4 Analysis by completion year for all CAR-T cell therapy trials

We analyzed the primary completion years of all the CAR-T cell therapy trials. We observed that 192 (Phase 1 - 123, Phase 2 - 56, Phase 3 - 1 and Other Phases - 12) CAR-T cell therapy trials are expected to be completed by 2021 followed by 159 (Phase 1 - 83, Phase 2 - 62, Phase 3 - 5 and Other Phases - 9) trials in 2022 (Figure 15). With such a huge number of trials expected to complete in the next couple of years, researchers would look forward to their results to gain valuable insights regarding the effect of both CAR-T design and clinical trial design on efficacy and safety from these therapies.

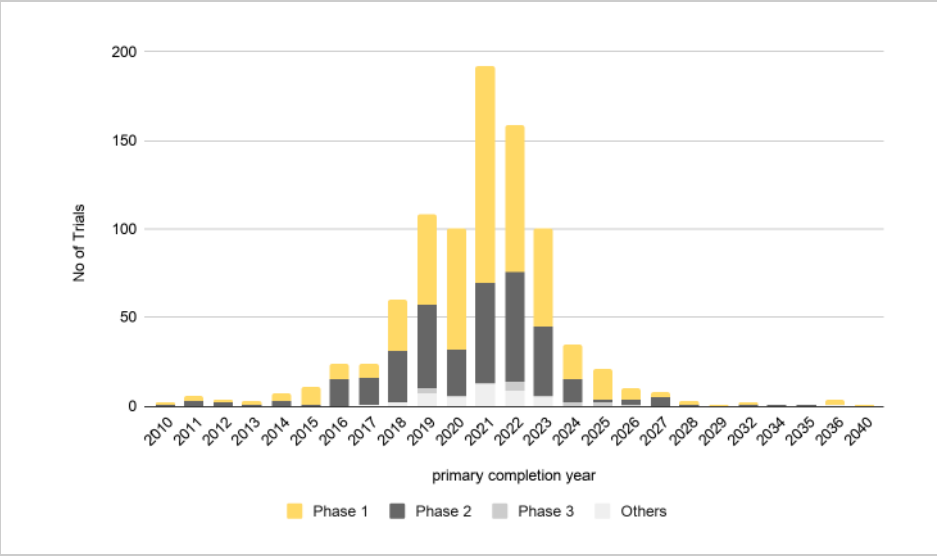


Figure 15 : Distribution of CAR-T cell therapy trials by primary completion year and phase

3.6 Route of Administration

CAR-T cell therapies are mainly administered via intravenous/infusion routes (93% trials (n= 829)) with other routes (3%) like intratumoral, intraperitoneal, intracerebroventricular, etc. being less used. Intravenous CAR-T cell therapies are most common and cover 59% of the trial population.

3.7 CAR-T cell therapy types

There were 608 studies (68%) that investigated autologous CAR-T cells, 62 (7%) allogeneic, 10 (1%) mixed, and 208 (24%) did not report the cell type (Figure 16). Majority of the studies have autologous cell types irrespective of the condition in the study. All the approved CAR-T cell therapies till date are autologous therapies. Allogeneic CAR-T cell types, or “off-the shelf” therapies, are still in initial stages of development with very few clinical trials that have results.

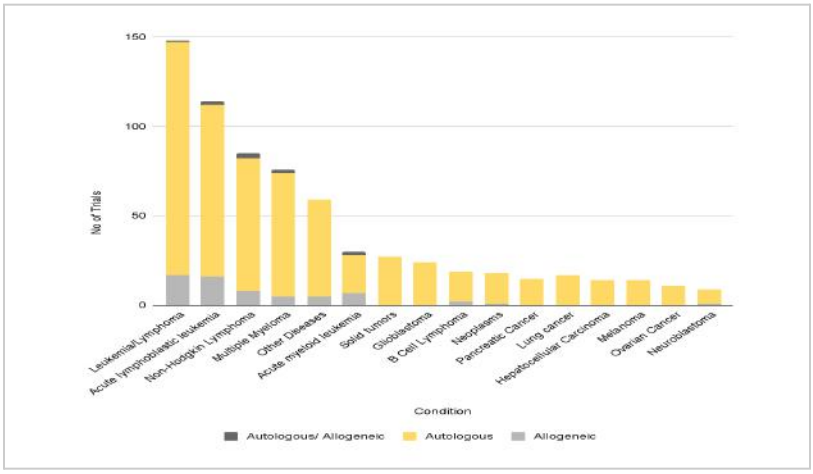


Figure 16: Distribution of CAR-T cell therapy trials by cell type and condition

3.8 Target antigen analysis

For most of the CAR-T cell therapy trials (n= 601), it is observed that CD19 is a widely used target antigen because it is expressed throughout the B-cell developmental phase to terminal differentiation into plasma cells[34] (Figure 17). For solid tumors, which generally do not express one tumor-specific marker, CAR-T cells identify tumor associated antigens such as CEA, HER-2, EGFR, GD2, mesothelin, MUC1 etc. This is evident from the graph below where increasing use of the TAAs mesothelin, NY-ESO-1, GD2, CEA etc. are seen in trials of recent years (Figure 17).

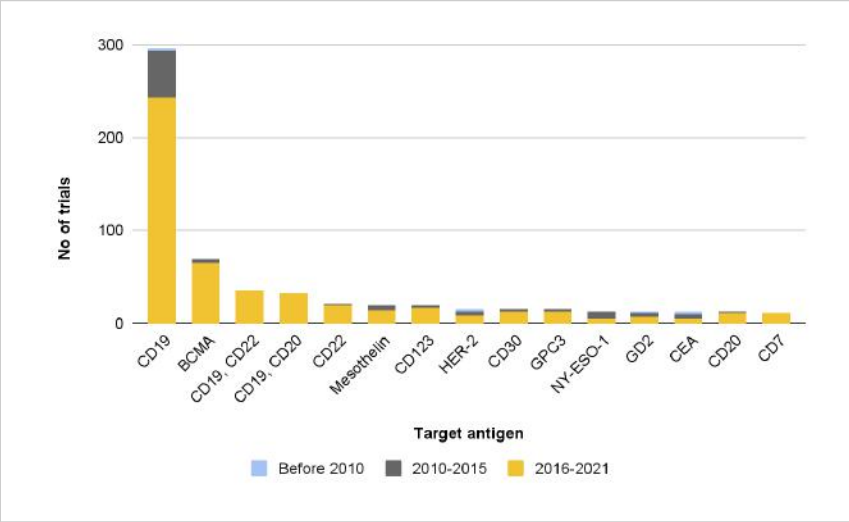


Figure 17 : Distribution of CAR-T cell therapy trials by target antigen and start year

3.8.1 Target Antigens v/s Conditions

In this subsection, we analyzed the most frequent target antigens in cell therapy across different conditions. Of 888 clinical trials, 389 studies (44%) explored CD19, 487 (54%) targeted alternative antigens and 12 (2%) studies did not mention target antigen used. Trials for Leukemia/Lymphoma, Acute lymphoblastic leukemia, Non-Hodgkin Lymphoma, and B Cell Lymphoma majorly investigated CD19 as a target antigen (Figure 18). BCMA as a target antigen is studied in multiple myeloma trials.

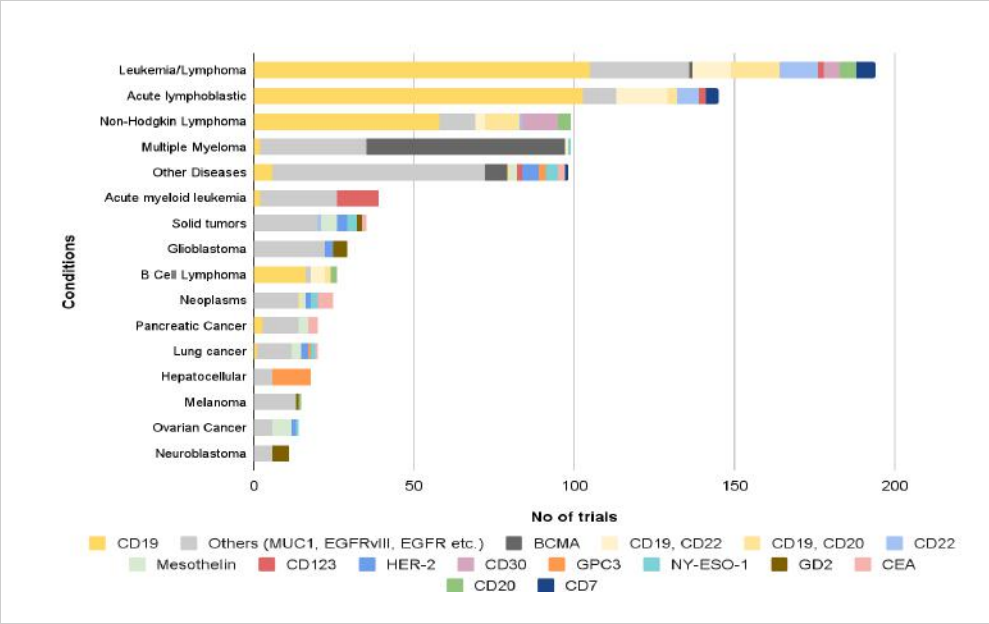


Figure 18 : Distribution of CAR-T cell therapy trials by target antigen and condition

3.8.2 Target Antigens v/s Type of Tumors

Studies with hematological malignancies explored CD19 (n = 288) as a target antigen majorly while solid tumors studies investigated non CD19 target antigens such as Mesothelin, HER-2, GPC3, GD2, CEA, NY-ESO-1 etc (Figure 19).

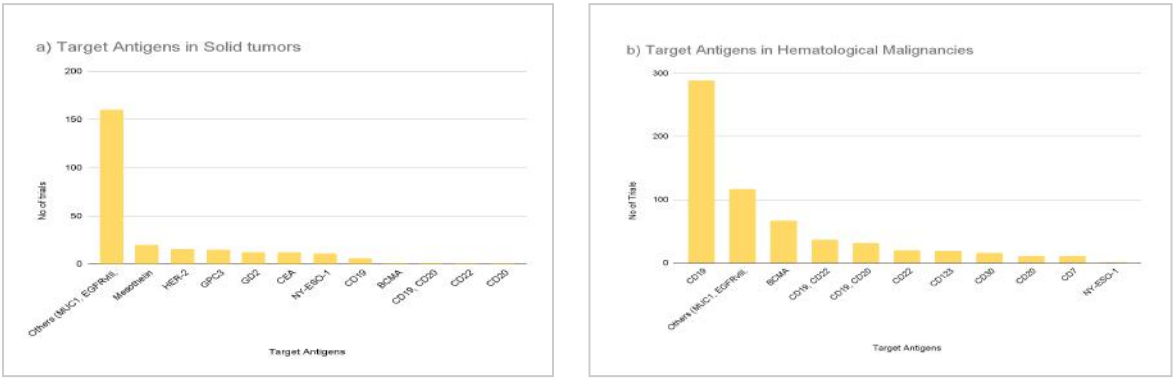


Figure 19 : Distribution of CAR-T cell therapy trials by type of tumor (a) Solid Tumors (b) Hematological malignancies and target antigens

3.9 Combination of Immune Checkpoint inhibitors with CAR-T Cells

Clinical trials (n= 15) for combination of CAR-T cells with Checkpoint inhibitors (ICI) are mainly studied in Neoplasms, B-cell Lymphoma, Gastrointestinal cancers, etc. Below table summarizes combination of CAR-T cells with ICI, with their targets and respective conditions under clinical studies with Pembrolizumab targeting PD-1 and Durvalumab targeting PD-L1 being the most studied (Table 5).

Checkpoint blockade	CAR-T cell/ TCR	Target	Condition	No. of trials
Pembrolizumab	Letetresgene autoleucel	PD-1	Neoplasms	2
Pembrolizumab	TCR-Transduced peripheral blood lymphocytes	PD-1	Metastatic HPV-16 Positive Squamous Cell Anal Cancer	1
Pembrolizumab	TCR-Transduced peripheral blood lymphocytes	PD-1	Endocrine Tumors, Non-Small Cell Lung Cancer, Ovarian Cancer, Breast Cancer, Gastrointestinal/Genitourinary Cancer, Neuroendocrine Tumors	1
Pembrolizumab	Tisagenlecleucel	PD-1	Diffuse Large B-cell Lymphoma	1
Pembrolizumab	TCR-transduced T-cells	PD-1	Malignant Epithelial Neoplasms	1
Pembrolizumab	iCasp9M28z T	PD-1	Malignant Pleural Disease, Mesothelioma, Metastases, Lung Cancer, Breast Cancer	1
Pembrolizumab	iC9-GD2 T Cells	PD-1	Neuroblastoma	1
Pembrolizumab	CART-EGFRvIII T-cells	PD-1	Glioblastoma	1
Ipilimumab	IL13R alpha2 CAR-T cells	CTLA-4	Recurrent/Refractory Glioblastoma	1
Ipilimumab	CD19CAR-28-zeta T cells	CTLA-4	B-Cell Lymphoma, Chronic Lymphocytic Leukemia, Acute Lymphocytic Leukemia	1
Durvalumab	JCAR017	PD-L1	Non-Hodgkin Lymphoma, Diffuse Large B-Cell Lymphoma, Follicular Lymphoma	1
Durvalumab	JCAR014	PD-L1	Diffuse Large B-Cell Lymphoma, Recurrent/ Refractory Diffuse Large B-Cell Lymphoma	1
Nivolumab	NeoTCR-P1	PD-1	Solid Tumor	1
Atezolizumab	KTE-C19 CAR-T cells	PD-L1	Refractory Diffuse Large B Cell Lymphoma	1

Table 5: Combination of Immune Checkpoint inhibitors with CAR-T Cells

3.10 Endpoint profile in CAR-T cell therapy trials

In this subsection, we have presented data for the CAR-T cell therapy trials where the trials met or did not meet their primary endpoint. For endpoint analysis, we have looked at only those trials (n= 120) which have results published on clinical trials registry, publications etc.

1. By phase and start year

For 120 trials analysed which met their primary endpoints, a spike in Phase 1 trials is seen in recent years while trend for Phase 2 trials is steep in the last decade.

2. By target antigens

Phase 1: Most trials with target antigen as BCMA met their primary endpoint followed by CD19, CD19-CD22 and NY-ESO-1.

Phase 2: Higher number of trials with CD19 as target antigen met their primary endpoint followed by HER-2.

3. By CAR-T cell generations and phase

Phase 1: More number of trials with third generation CAR-T cells met their primary endpoints followed by second and fourth generation.

Phase 2: Trials with second generation CAR-T cell therapies met their endpoints more frequently followed by third generation cell therapies.

4. By signaling domain

Phase 1: Higher success rate, defined as meeting primary endpoint, was observed for trials with signaling domain CD28, 4-1BB followed by CD3-zeta, 4-1BB, CD28 and CD3-zeta, CD28.

Phase 2: High success rate was observed for trials with signalling domain CD3-zeta, 4-1BB, followed by CD3-zeta, 4-1BB, CD28.

5. By Cell Type

Phase 1 and phase 2 trials with autologous cell therapies have a higher rate of success compared to allogeneic cell types.

6. By Target Organ

Trials with target organs as lymphatic system and blood cells showed higher percentage of success rate than breast, brain and liver

7. By Condition

High success rate was observed for the following conditions:

Phase 1: Multiple Myeloma followed by Leukemia/Lymphoma and Solid tumors

Phase 2: Non-Hodgkin lymphoma followed by Leukemia/Lymphoma and acute Lymphoblastic Leukemia

3.11 Adverse Events

In this section we have a deeper look at occurrence of adverse events in CAR-T cell therapy trials by reviewing data published in clinical trials registries mostly for conditions such as Leukemia/Lymphoma, Melanoma, Multiple Myeloma and Acute Lymphoblastic Leukemia.

3.11.1 Top Serious Adverse Events

This subsection lists the most common serious adverse events observed in patients who participated in CAR-T cell therapy trials (Table 6).

Serious Adverse Event Type	No. of subjects affected	No. of subjects at risk	% patients affected
Cytokine release syndrome	187	389	48%
Febrile neutropenia	98	387	25%
Hypotension	73	439	17%
Encephalopathy	67	408	16%
Pyrexia	62	368	17%
Hypoxia	46	437	11%
Seizure	22	343	6%
Acute kidney injury	21	338	6%
Somnolence/depressed level of consciousness	18	76	24%
Sepsis	17	326	5%

Table 6: Top serious adverse events based on the number of patients affected

3.11.2 Solid Tumors v/s Hematological Malignancies

Table 7 shows the percentage of patients in which serious adverse events and other adverse events were observed by type of tumor.

Type of Tumor	Total SAEs	Total OAEs
Hematological malignancies	66% (413/630)	94% (592/630)
Solid tumors	21% (47/225)	95% (214/225)

Table 7: Serious and other adverse events by type of tumor

Based on the data, CAR-T cell therapies show less serious adverse events in studies conducted for solid tumors compared to hematological malignancies.

4. Summary and key takeaways

CAR-T cell therapies have the potential to revolutionize the therapeutic approach addressing solid tumors and hematological malignancies. Providing benefits for many years and targeted CAR-T cells being “live cells”– they can amplify in the patient to establish immune memory, provide continuous surveillance to treat local and metastatic lesions. Although CAR-T cell therapies have shown clinical responses, the challenges such as severe toxicities, modest anti-tumor activity, antigen escape, restricted trafficking and limited tumor infiltration limit their therapeutic efficacy. However, promising data has also

been obtained from some CAR-T cell therapies that further validate the use of this therapy as a potential treatment:

- Follow-up data for axicabtagene ciloleucel induced durable responses and a median overall survival of greater than 2 years with long-term safety profile in patients with relapsed or refractory large B-cell lymphoma[35]
- Lisocabtagene maraleucel resulted in a high objective response rate, with a low incidence of adverse events in patients with relapsed or refractory large B-cell lymphomas[36]
- A phase 2 trial of Kymriah demonstrated 82% of the patients achieved complete remission with incomplete blood count recovery within three months of infusion[37]

Majority of clinical trials using CAR-T cells are early phase studies in B cell malignancies, with a dramatic increase in trial numbers in 2016 which continued at a rate of more than 100 new trial registrations each year. CAR-T cell therapy focused companies have witnessed a surge in M&A with three times higher market growth since 2017-18 till now. Around 13 deals amounting to ~$15 billion shows gradual establishment of CAR-T cell therapeutics industry along with increased investors' interest.

Our research on the pipeline of CAR-T therapy segment led us to following takeaways:

- Corpus of 888 trials of adoptive cell therapies including CAR-T cells, CAR-NK cells and TCR cells showed 83% trials have been initiated after 2016 and 7% trials have completed their studies while 53% trials are still recruiting patients
- Only a few Phase 3 trials have been initiated since 2016, reflecting the difficulty of early stage clinical trials to meet safety/efficacy endpoints
- CAR-T cell therapy is mainly studied in oncological indications *viz.* acute lymphoblastic leukemia, non-Hodgkin lymphoma, multiple myeloma, acute myeloid leukemia and other hematological malignancies. Initial success in these have led to study in solid tumors like glioblastoma, pancreatic cancer, lung cancer, hepatocellular carcinoma and melanoma
- Non-oncological conditions include HIV Infections, CD19 Positive Systemic Lupus Erythematosus, Neuromyelitis Optica Spectrum Disorders, POMES Syndrome, etc.
- CD19 is the most widely used target antigen in hematological malignancies, TAAs in solid tumors include mesothelin, NY-ESO-1, GD2, CEA etc.
- Pembrolizumab targeting PD-1 and Durvalumab targeting PD-L1 are mostly studied as combination therapies of CAR-Ts cells with immune checkpoint inhibitors
- Novartis, Celgene and Gilead Sciences are the top industry sponsors which have advanced pipelines with trials in Phase 3
- More than 25% patients have adverse events from CAR-T therapies such as Cytokine release syndrome and Febrile neutropenia

The clinical success and landmark approvals has opened new and encouraging avenues for developers of adoptive cell therapies. Research in the field of CAR-T cells has progressed rapidly. Newer techniques of CAR-T cell engineering using gene editing tools like CRISPR and TALEN are focussed on optimizing the efficacy of the engineered T cells as well as reducing the associated adverse events. Research is also ongoing to make these therapies more effective in solid tumors. Development of effective allogeneic

CAR-T cells, with limited potential for graft-versus-host disease, would make this therapy more cost effective and quickly accessible to needy patients, even with impared T cell activity. With several such advances in the pipeline, CAR-T cell therapy is slated for major breakthroughs in the treatment of cancer.

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