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CAR-T cells, du principe aux applications cliniques

CAR-T cells, from principle to clinical applications

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ABSTRACT

Chimeric antigen receptors (CAR)-T cells are genetically engineered T-lymphocytes redirected with a predefined specificity to any target antigen, in a non-HLA restricted manner, therefore combining antibody-type specificity with effector T-cell function. This strategy was developed some thirty years ago, after extensive work established the key role of the immune system against cancer. The first-engineered T-cell with chimeric molecule was designed in 1993 by Israeli immunologist Zelig Eshhar. Since then, several modifications took place, including the addition of co-stimulatory domain, to further improve CAR-T cell anti-tumor potency. The first clinical application of CAR-T cell was done in Rotterdam in 2005 for metastatic renal cell carcinoma and simultaneously at the National Cancer Institute (NCI) for metastatic ovarian cancer. These pioneered studies failed to demonstrate a therapeutic benefit, but warning emerged concerning their safety of use. The real clinical success came with anti-CD19 CAR-T cells, used since 2009 by Steven Rosenberg at the NCI in a patient with refractory follicular lymphoma and in 2011 by Carl June and David Porter from the University of Pennsylvania in patients with chronic lymphocytic leukemia and B-cell acute lymphoblastic leukemia. From that time, large centers in North America have embarked in several early phase and pivotal trials that have demonstrated unprecedented response rate in heavily pretreated chemo refractory patient with B-cell malignancies. These clinical success have led to the approval of three anti-CD19 CAR-T cells products for the management of B-cell malignancies in the United States and in Europe as of December 2020.

KEYWORDS : *Immunotherapy, cell therapy, CAR-T cells*

RÉSUMÉ

Les lymphocytes T porteurs d'un récepteur à l'antigène chimérique (CAR-T) sont des cellules génétiquement modifiées pour combiner une reconnaissance antigénique spécifique et non HLA-dépendante aux fonctions effectrices des lymphocytes T. Cette stratégie a été développée il y a environ 30 ans après que de nombreux travaux aient démontré le rôle anti-tumoral du système immunitaire. Les premiers CAR-T ont été conçus par l'immunologiste Israélien Zelig Eshhar. Depuis, de nombreuses modifications ont eu lieu, dont l'ajout de signaux de co-stimulation, afin d'améliorer l'efficacité anti-tumorale de ces cellules. La première application clinique des CAR-T a été réalisée à Rotterdam en 2005 chez des patients atteints de cancer rénal métastatique, et au National Cancer Institute (NCI) pour des cancers ovariens métastatiques. Ces études pionnières n'ont pas réussi à démontrer l'intérêt thérapeutique des CAR-T mais ont souligné leurs effets indésirables. Le véritable succès a eu lieu grâce aux CAR-T anti-CD19, utilisés dès 2009 par Steven Rosenberg au NCI chez un patient atteint de lymphome folliculaire réfractaire, puis en 2011 par Carl June et David Porter de l'Université de Pennsylvanie chez des patients atteints de leucémie lymphoïde chronique et leucémie aigue lymphoblastique B. Dès lors, de grands centres Nord-Américains ont conduit de nombreux essais cliniques ayant démontré des taux de réponses sans précédent chez des patients multi traités atteints d'hémopathies malignes B. Ces succès cliniques ont permis l'autorisation de mise sur le marché de trois produits CAR-T pour le traitement des hémopathies malignes B aux États Unis et en Europe.

MOTS CLEFS : *Immunothérapie, thérapie cellulaire, CAR-T*

INTRODUCTION

Since the emerging concept of cancer immunotherapy in the 1960s, extensive research has been performed to redirect immune system effector cells against tumor cells. In the late 1980s, early steps of adoptive T-cell therapy paved the way for T cells redirected to specific antigen target through engineered chimeric antigen receptors (CAR), with the first CAR design published in 1993 by Israeli immunologist Zelig Eshhar. CAR are recombinant receptors which redirect T-cell function to a specific antigen expressed at the tumor cell surface. Many new engineering strategies have been used since then to improve CAR-T cells potency. Successful results in recent trials have been observed in chemo refractory aggressive B-cell lymphoma and B-cell acute lymphoblastic leukemia (B-ALL), leading to their approval in the United States and Europe since 2017. These autologous adoptive cell therapies are emerging as powerful therapies in B-cell leukemia and lymphoma and major efforts are being made to translate them in other subtypes of hematological malignancies and in solid tumors. In this review, we provide a comprehensive historical and biological perspective regarding the advent of CAR-T cell therapies, with an emphasis on fundamental discovery of antitumor immunity and T-lymphocyte potency. We present the evolution of CAR engineering through several generations and discuss future directions. Finally, we highlight results from early and more recent pivotal clinical trials that demonstrated therapeutic efficacy associated with CAR-T cells.

PRINCIPLE OF CAR T-CELLS DEVELOPMENT

Cancer immunotherapy using T cells has long been of interest and it took a century from the first recognition of anticancer immunity to the first CAR-T cell. The **Figure 1** summarizes the key milestones in history of CAR-T cell development, from basic research to first clinical applications and pivotal trials leading to Food and Drug Administration (FDA) and European Medicines Agency (EMA) approvals.

[History of anticancer immunity](#)

The first significant recognition of the potential role of the immune system to fight cancer originated in the nineteenth century with New-yorker surgeon William Coley. In the early 1880s, a German immigrant presented with an inoperable neck tumor that vanished after he developed an erysipelas, a superficial skin infection most commonly caused by *Streptococcus pyogenes*. Interested in the reason for that patient's recovery, Coley investigated several similar cases and hypothesized that bacterial infections may cause tumor regression. He then systematically treated with a mixture of heat-killed *Streptococcus pyogenes* and *Serratia marcescens* (also known as "Coley's Toxin") his own patients and observed the tumor shrink[1]. His study published in 1893 yielded variable results but with some clear clinical successes, particularly against bone and soft tissue sarcoma. This was one of the first example of anti-cancer immunotherapy. However, these works were controversial because being anecdotal, lacking rigor and difficult to repeat, and the concomitant development of radiotherapy and chemotherapeutic agents prevented further explorations in this field.

The concept of cancer immunotherapy reemerged in the early twentieth century. In 1909, Paul Ehrlich hypothesized for the first time that neoplastic cells are constantly generated in the body but eradicated by the immune system. This "immunological surveillance" hypothesis was further developed by Sir Frank MacFarlane Burnet and Lewis Thomas later speculated that the immune system can recognize and target tumor-associated "neo-antigens"[2].

In 1961, immunologist Jacques Miller characterized T lymphocytes and identified the thymus gland as the place where they develop[3]. By contrast with B cells that recognize antigen in its native form, T cells recognize peptide antigens, derived from proteins degraded intracellularly, that are loaded onto cell surface major histocompatibility complex (MHC) molecules. T cells are characterized by a unique cell surface receptor, called the T-cell receptor (TCR), that was first discovered in 1982 by Nobel laureate James P. Allison[4]. The TCR is composed of an antigen-recognizing unit made of highly polymorphic single α and β chains (for the majority) that contain variable and constant regions, associated with the signaling transduction chains CD3 γ , δ , ϵ and ζ . Antigen stimulation of the TCR is necessary for T-cell activation and proliferation, but an additional second signal of co-stimulation is required for full T-cell potency. The surface protein CD28 and its family members are the most potent co-stimulatory receptors on T cells, that recognize B7-1 and B7-2 expressed on antigen-presenting cells, but many other exists (CD137 or 4-1BB, OX40, ICOS, CD27)[5]. Therefore, the T lymphocyte, with its capacity for antigen-directed cytotoxicity, gradually imposed as a central weapon for engaging the immune system in the fight against cancer. Various anticancer therapeutic approaches now exploit T-cell functions that include cytokines to boost T-cell potency, checkpoint blockage to release T-cell response, cancer vaccines to elicit a T-cell response, T-cell redirecting bispecific antibodies and adoptive T-cell therapies based on infusion of target-specific immune cell.

[Adoptive T-cell transfer therapies](#)

Adoptive T-cell therapy (ACT), in which autologous or allogenic T cells are infused into patients with cancer, has shown considerable promise in recent years. Allogeneic hematopoietic stem cell transplantation (allo-HSCT) for leukemia represents the earliest adoptive transfer of T cells with anti-cancer activity developed in the 1950s[6]. The first success reported of ACT was shown in 1966 by Chester Southam from the Memorial Sloan Kettering Cancer Center (MSKCC) when half of the 41 patients with advanced cancer demonstrated tumor regression following co-transplantation of patient-derived leukocytes with autologous tumor cells[7]. Two types of ACT currently exist: tumor-infiltrating lymphocytes (TILs) and engineered T cells.

Tumor-infiltrating lymphocytes

The earliest trial using lymphocytes isolated from cancer samples was pioneered by Steven Rosenberg at the surgical branch of the National Cancer Institute (NCI) in 1988. His team isolated TILs from patient with metastatic melanoma, expanded them in vitro and re-infused them with interleukin-2 (IL-2). He observed tumor regression in 11 out of 20 patients[8], and in 34% (29/86) of patients in a larger cohort later reported in 1994. However, only 5 of the 29 responses were complete, and the median duration of response was only 4 months[9]. A major breakthrough occurred when Rosenberg incorporated lymphodepleting regimen (cyclophosphamide and fludarabine with 0, 2 or 12

gray total body irradiation) prior to autologous cell transfer in 93 patients with metastatic melanoma. The addition of lymphodepletion increased response rates to 49%, 52%, and 72%, respectively. Complete tumor regression was achieved in 20 of the 93 patients treated (22%), 19 of whom persisted beyond 3 years[10]. Despite these promising results, TILs present key limiting factors : (1) most of cancer lack TILs (and are so-called “cold tumors”)[11] or they cannot be harvested from them or cultured ; (2) TILs have a Human-Leukocyte Antigen (HLA)-restricted specificity but tumors often silence their MHC as part of immune escape mechanisms ; (3) many tumors do not express costimulatory ligand that are required for full T-cell antitumor activity and (4) tumor immunosuppressive microenvironment often induce T-cell exhaustion or dysfunction[12]. In order to overcome these limitations, autologous lymphocytes genetically engineered to target a specific cancer antigen in an MHC-independent manner were developed.

Engineered T lymphocytes or “T-bodies”

“T-bodies” are genetically engineered T cells armed with antigen-specific chimeric receptors that combine antibody non-HLA restricted specificity through an extracellular antibody-derived recognition domain with T-cell effector function through intracellular lymphocyte-stimulating moiety(ies). “T-bodies” were originally designed to better understand TCR activation and antigen interaction, but their potential for cancer immunotherapy rapidly emerged. Unlike TCR, chimeric receptor provide a more universal approach as they engage surface antigens in their native form that do not require peptide processing or HLA expression to be recognized and they offer broader range of potential targets (protein, carbohydrate and glycolipid structures)[13].

TCR-modified T-cells

The first successful engineered T cells with an immunoglobulin (Ig)-TCR-chimeric molecule was pioneered by Israeli immunologists Zelig Eshhar and Gideon Gross in 1989[14]. They replaced the variable regions (V α and V β) of the TCR chains with their variable heavy (VH) and light (VL) immunoglobulin homologs of an anti-2,4,6-trinitrophenol (TNP) antibody. These first T-bodies were capable of killing TNP-coated cells in a non-MHC-restricted manner. However, the production of these “double-chain” chimeric receptor required a double transfection procedure that was cumbersome. Therefore, simpler strategies based on single-chain fragment variable (scFv) were developed.

CAR-T cells

CARs are synthetic hybrid receptors formed by the fusion of an extracellular antigen-recognition domain consisting of a scFv, either derived from monoclonal antibodies or synthesized from human libraries, linked through a hinge sequence to the intracellular signaling domains of the T-cell coreceptor CD3 ζ , which contains three immunoreceptor tyrosine-based activation motifs (ITAMs), and various co-stimulatory signaling domain. This chimeric receptor is capable by itself to trigger T-cell activation and stimulate proliferation and effector functions including interleukin release and target cell killing upon antigen recognition.

EVOLUTION OF CAR-T CELLS ENGINEERING

Over the past 30 years, the evolution of CAR design has mainly involved their intracellular signaling domain, but also their extracellular domain mediating antigen recognition. The **Figure 2** summarizes the evolution from TCR to next generation CAR-T cells.

CAR signaling

The progress in the development of CAR signaling over the past three decades can be grouped into four CAR generations based on the structure and composition of the endodomain.

Inspired from their first TCR-engineered T-cells, Eshhar and colleagues engineered in 1993 the first CAR construct, using a scFv domain derived from an ovarian carcinoma-specific monoclonal antibodies, fused to the CD3 ζ signal transducing subunit of the TCR[15]. This first generation chimeric receptor was capable of specific lysis of human ovarian carcinoma cell line[16]. In the first preclinical trial, mouse T-cells expressing HER2-specific chimeric receptor were administered into nude mice together with HER2-expressing tumor, which led to delayed tumor development[17]. While innovative, this first generation of CAR relied solely on the CD3 ζ chain to simulate TCR signaling and showed low cytotoxicity and T-cell expansion. The lack of costimulatory elements limited their efficiency in clinical trials[18].

For optimal and sustained T-cells function, costimulatory domains have been added into T cells through the CAR construct. In 1998, Helene M. Finney described the first chimeric receptors providing both primary and costimulatory signaling (CD28-based) in T cells, with evidence for enhanced proliferation, cytokine secretion, survival and anti-

tumor activity[19]. In 2004, another chimeric receptor with 4-1BB signaling capacity elicited potent cytotoxicity against B-cell acute lymphoblastic leukemia (B-ALL) in vitro[20]. Second generations of CARs were designed as tripartite fusion receptor possessing both activating and costimulatory domain (including CD28, CD137 or 4-1BB, OX40 or ICOS) to obtain greater strength of signaling. The two costimulatory domains that have been used in clinic so far are CD28 and 4-1BB, that differentially influence the metabolic characteristics of CAR-T cells. The CD28 costimulatory domain favor intense and early proliferation of CAR-T cells after infusion, through B7 signaling pathway, with effector memory differentiation. In contrast, 4-1BB containing construct are slower to expand, through activation of the nuclear factor- κ B (NF- κ B) and tumor necrosis factor receptor-associated factor (TRAF) pathway, yielding CD8+ central memory T-cells, with enhanced persistence in vivo[21].

Third generation of CARs, encompassing two or more costimulatory signaling domains combined with an activating domain have emerged later. In 2010, Tammana and colleagues from the University of Pennsylvania (UPenn) developed a CAR containing CD28 and 4-1BB costimulatory moieties with improved effector functions and in vivo persistence[22], as well as comparable safety profile in limited phase I trial[23]. Other costimulatory molecules have been used in varied configurations, but exhaustive comparisons are still lacking.

Fourth generation CARs, also called TRUCKS (T-cells redirected for antigen-unrestricted cytokine-mediated killing) or armored CARs, combine a second-generation CARs backbone with a nuclear factor of activated T-cells (NFAT)-driven inducible cytokine (preferentially but not exclusively) expression module. After CAR-T cell binding to the tumor antigen, CAR signaling leads to NFAT phosphorylation, migration to the nucleus and induction of the NFAT-responsive promoter that drives transgene expression. The secreted cytokines, especially IL-7, IL-12, IL-15 or IL-18, not only prolong CAR-T cell activation and improve their killing activity, but also modulate the tumor microenvironment[24].

Recent improvements of CAR designs observed in in vitro or in animal models have not been further validated in patients, and proper comparison across the different CAR generations remains to be done.

[CAR targeting](#)

In principle, any cell surface molecule can be targeted through a CAR. However, one major limitation to the development of CAR-T cell therapies is the requirement for truly tumor-specific antigens that are expressed in tumor cells but absent in normal tissues. Expression of the targeted antigen in normal cells can result in severe toxicity, whereas absence or decreased antigen expression in tumor cells can lead to tumor escape. Although initial attempts to target CD19 with monoclonal antibody were unsuccessful, this pan-B cell antigen is to date the most widely investigated target for CAR-T cell therapy. CD19 is an ideal target owing to its uniform expression in the majority of B-cell malignancies and the dispensability of normal CD19 B cells for immune integrity that can be supplemented by immunoglobulins. CD19 was first used as a target in 2003 by Sadelain et al. when they demonstrated that human CD19-directed CAR-T cells can kill chronic lymphocytic leukemia (CLL) cells in tumor-bearing mice[25]. Others B-cell antigens (mainly CD20 and CD22) are under investigation in dual CAR design to overcome CD19-tumor escape. In the field of multiple myeloma (MM), B-cell maturation antigen (BCMA), emerged in 2015 with the development of monoclonal anti-BCMA antibody[26]. BCMA is a member of the tumor necrosis factor superfamily of proteins that is expressed by malignant and normal plasma cells and some mature B cells. Friedman et al. published in 2018 effective targeting of BCMA using CAR-T cells in mouse MM models[27]. As for T-cell malignancies, pan-T cell antigen (CD5 and CD7) or tumor restricted T-cell antigen (CD30 or TCR- β chain 1) are being evaluated in early phase trials, but fratricide in case of pan-T cell target and life-threatening T-cell aplasia remains major challenges to overcome. In solid tumor, despite an impressive number of investigated targets, few target candidates are strictly restricted to the tumor cells and clinical experiences have been less encouraging. Main targets under development include mesothelin, epidermal growth factor receptor (EGFR), GD2 or HER2[28].

[Next generation of CARs](#)

Innovative strategies to enhance the therapeutic outcome of CAR-T cells are being explored. These approaches mainly focus on (1) improving T-cell activation and persistence (TRUCKs, different cytokine culture condition, modification of transmembrane and hinge regions, additional costimulatory molecule), (2) overcoming tumor immunosuppressive microenvironment (knockout of PD1, CTLA4 or LAG3) and (3) impaired T-cell trafficking to the tumor, and (4) addressing tumor escape (bispecific CAR, dual CAR or tandem CAR). In addition, various T-cell subsets (invariant NK-T cells (iNKT), mucosal-associated invariant T cells (MAIT)) and other immune cells, such as natural killer (NK) cells, are being investigated for CAR-T cells therapy.

[Allogenic CAR-T cells](#)

Another hurdle for CAR therapy is the long manufacturing process, with difficulty for most patients with end-stage chemo refractory disease to wait up to 5 weeks before infusion. Therefore, several efforts have been focused on

developing “off-the-shelf” ready-to-use CAR-T cells from third party healthy donors. Such allogenic CAR-T cells can be generated from peripheral mononuclear blood cell (PMBC) of healthy donor, but they could also derive from umbilical cord blood (that may be associated with decrease alloreactivity) or induced pluripotent stem cell. The use of allogenic CAR-T cells presents many potential advantages over autologous approaches, including: (1) immediate availability, (2) redosing or combination of CAR-T cells directed against different target, (3) improved T-cell function (which, compared to autologous T-cells, have not been negatively impacted by previous lines of chemotherapies or evolved in an immunosuppressive environment) and (4) lower production cost. However, allogenic CAR-T cell present with two major issues: first, the risk of life-threatening graft-versus-host disease (GVHD), and second the rapid elimination of allogenic T-cells by the host immune system limiting their persistence and efficacy. The first issue can be overcome using virus-specific T-cells (such as EBV-specific T-lymphocytes) with restricted repertoire limiting alloreactivity, non-conventional $\alpha\beta$ -T cells (such as $\gamma\delta$ -T cells that activated their TCR in an MHC-unrestricted manner, iNKT or MAIT cells with a restricted TCR repertoire or innate NK cells) or using TCR-edited T-cells. As for allorejection, the use of HLA-matched CAR-T cells is being investigated, as well as the modification of the conditioning regimen with addition of anti-CD52 monoclonal antibody alemtuzumab to extend the duration of lymphopenia and improve CD52-edited CAR-T cells engraftment[29]. The development of next-generation allogeneic CAR-T cells to address these issues is a very active area of research.

CLINICAL APPLICATION OF CAR-T CELL THERAPY

Proof of concept for the therapeutical benefit of cancer-specific CAR-T cells has been provided in vitro and in animal models, leading to early phase trial in the early 2000s.

Early clinical trials

Solid tumors

The first CAR introduced in a cancer patient was done by Lamers et al. from Netherland in 2005. They used engineered CAR-T cell against carbonic anhydrase IX (CAIX) in 12 patients with CAIX-expressing metastatic renal cell carcinoma (RCC). Patients were treated with a maximum of 10 infusions of 0.2 to 2.10^9 CAR-T cells. Toxicity grade 2-4 of liver enzyme occurred at the lowest CAR-T cell dose, necessitating treatment cessation in 4 out of 8 patients. Liver biopsies revealed the destruction cells of normal bile duct epithelial cells, expressing CAIX, by CAR-T cells. It was a striking example of an “on-target, off-tumor” toxicity and a reminder that CAR strategies need to further guarantee targeting specificity[30]. In parallel, another phase I study was conducted by Kershaw et al. from the NCI to assess the safety and efficacy of CAR-T cells directed against the ovarian-associated antigen alpha-folate receptor (FR) for the treatment of metastatic ovarian cancer. Eight patients in cohort 1 received a dose escalation of CAR-T cells in combination with high-dose IL-2. Treatment-related toxicity grade 3-4 were observed in 5 patients in the first cohort, probably due to IL-2 administration. However, no reduction in tumor burden was seen in any patient, possibly due to the lack of CAR-T cells localization to the tumor and their quick decline within one month after infusion[31]. These pioneered studies brought little evidence of therapeutic benefit of CAR-T cells in oncology but warning concerning their safety.

Hematological malignancies

The real breakthrough of CAR therapy came in the second decade of this century, when several large cancer centers in North America embarked on treating B-cell lymphoma and leukemia. Hematological malignancies were the perfect model for CAR strategy, given the uniform expression of the CD19 antigen and the natural T-cell homing to hematological organs.

B-cell lymphomas

The first group to report on significant responses in B-cell lymphoma patients was led by Steven Rosenberg and James Kochenderfer at the NCI. In 2009, they treated a patient with chemo refractory follicular lymphoma (FL) using CD19-CD28 ζ CAR-T cells, that resulted in dramatic regression of tumor infiltration[32]. This successful result led to a phase I trial including 15 patients with relapsed-refractory (R/R) diffuse large B-cell lymphoma (DLBCL) and indolent B-cell malignancies. After a lymphodepleting chemotherapy (cyclophosphamide and fludarabine, Cy/Flu), 5.10^6 CAR T-cells/kg were re infused. Twelve of the 13 evaluable patients came with objective response rate (ORR) (92%) and 8 patients had complete remission (CR) (67%). Toxicities mainly included cytokine release syndrome (CRS) and neurotoxicity, and led to reduced dose of CAR-T cells from 5 to 1.10^6 T cells/kg during the study[33]. In 2017, results were updated reporting sustained remission (from 38 to 56 months) in 4 of the 5 evaluable DLBCL patients with initial

CR, suggesting the curative potential of this strategy, while dealing with heavily pretreated patients. Interestingly, in 3 of these patients, recovery of CD19+ polyclonal B-cell took place while they all remained in CR[34].

The second group to report on substantial improvement in hematological malignancies was led by Carl June and David Porter from UPenn. In 2011, they reinfused CD19-specific 4-1BB ζ CAR-T cells to a patient with refractory CLL. They observed remissions ongoing for 10 months after treatment, along with CAR-T cells persistence at high level for 6 months in the blood and bone marrow[35]. From 2014 to 2016, they treated 28 patients with the same CAR construct (14 DLBCLs and 14 FLs). A CR was observed in 6 of the 14 DLBCL patients (43%) and in 10 out of the 14 FL patients (71%). A severe CRS occurred in 5 patients (18%) and severe neurotoxicity in 3 patients (11%), one being lethal. After a median follow up of 29 months, all patient with CR after 6 months remained in remission. Half of the patients experienced recovery of normal circulating B-cells with a median of 29 months after therapy[36].

The Fred Hutchinson group led by Cameron Turtle, David Maloney and Stanley Riddell in Seattle reported in 2016 the experience of 32 heavily pretreated patients with different sub types of B-cell non-Hodgkin lymphomas (NHLs), including 11 DLBCL, 11 transformed-indolent lymphoma, 6 FL and 4 mantle cell lymphomas (MCL). Based on previous work showing that CAR-T cell products generated from defined T cell subset provided synergistic antitumor effect in mouse model[37], the group evaluated a defined ratio of CD8/CD4 CD19-specific 4-1BB ζ CAR-T cells. Each T-cell subset was transduced separately, then re infused together in a 1:1 CD4/CD8 ratio. In this trial, lymphodepletion chemotherapy included Cy with or without Flu, and they observed that Cy/Flu lymphodepletion increased CAR-T cell expansion and persistence, and response rates (72% ORR and 50% CR) compared to Cy-based lymphodepletion alone (50% ORR, 8% CR). The author demonstrated that Cy/Flu, through an optimal lymphodepletion, limited the immune response mediated by residual T cells against the murine scFv component of the CAR, responsible for improved CAR-T cell expansion and clinical efficacy. Severe toxicities (CRS and neurotoxicity) were observed in 13 and 28% of the patients respectively, due to high dose of CAR-T cells re infused (2.10⁷/kg). Finally, this study identified that early serum biomarker elevation correlated with subsequent severe CRS (IL-6, IFN-gamma, IL-15, IL-8, and IL-10) and neurotoxicity (IL-6 and IL-15)[38]. As shown in **Table 1**, these first studies established the basics of the use of CAR-T cells in hematological malignancies in term of dosage, optimal lymphodepleting regimen, sides effects and efficacy.

Acute B-cell lymphoblastic leukemia

In the field of B-ALL, the real success was achieved in 2012 by Stephan Grupp and colleagues from the Children's Hospital in Philadelphia (CHOP). They reinfused 2 children with R/R B-ALL with anti-CD19 CAR-T cells. Complete remission was observed in both patients, but one had early CD19-negative relapse 2 months after treatment[39]. The other patient, a 7-year-old girl named Emily Whitehead, who was the first children receiving CAR-T cells, experienced all severe complications of this therapy, including life-threatening CRS with pressor and ventilatory support, but was finally cured. Today, Emily is more than 8 years free from cancer. The same team led a pilot trial in 2014 in 25 children and 5 young adults with R/R B-ALL receiving CD19-4-1BB ζ CAR-T cells. Morphological CR was achieved in 27 patients (90%), 73% of whom obtained minimal residual disease (MRD)-negative CR as assessed by flow cytometry[40]. The **Table 2** lists major early phase trials of CD19 CAR-T therapy in patients with R/R B-ALL that have established the safety and efficacy of this strategy. These trials, despite variation in CAR constructs and manufacturing, patient population and dosing schemes, have consistently shown unprecedented CR rates in high-risk, heavily pretreated patients with R/R B-ALL. However, the main challenge in B-ALL remains difficulty in obtaining durable responses. Despite initial high rate of CR obtained with CAR-T cell therapy, more than half of adult B-ALL patients relapse if not bridged to allo-HSCT[41,42].

Acute myeloid leukemia

As for acute myeloid leukemia (AML), the main challenge limiting the use of CAR therapy is the lack of AML-specific antigen, with most myeloid antigens often co-expressed on normal HSCP and malignant blasts, exposing to myeloablation that would require rescue allo-HSCT. The first clinical trial to demonstrate an activity of CAR-T cells was published in 2013 by Ritchie et al. using a CAR targeting the Lewis Y antigen. Although all 4 patients eventually progressed after limited efficacy (2 stable disease, 1 partial response and 1 cytogenetic response), this study was important as it demonstrated biological activity of CAR-T cells against AML without significant hematopoietic toxicity[43]. Currently, most of the CAR trials conducted in AML patients target CD33, CD123 or CLL-1. CD33 and CD123 are appealing target antigens, almost ubiquitously expressed on AML blasts, but also by normal hematopoietic stem and progenitor cell (HSPC)[44]. Wang et al. reported in 2013 a partial response to CD33-specific CAR-T cells in a 41-year old patient with refractory AML, with transient reduction of AML blasts in the marrow before disease progression 9 weeks after CAR-T cell infusion[45]. For CD123, the phase I trial assessing Cellectis' universal CAR-T cell UCART123 was suspended in 2017 after one patient died from CRS and capillary leak syndrome (CLS). It has later been reported that CD123 was expressed on the endothelium of small-caliber blood vessels, raising concern of additional

toxicity in the form of vascular leak[46]. In order to mitigate this toxicity, the UPenn group designed biodegradable CD123 CAR-T cells manufactured via mRNA electroporation rather than lentiviral transduction[47]. Though there was no measurable anti-tumor response, the favorable safety profile, with absence of vascular toxicity, paved the way for a phase I trial of lentivirally-transduced second-generation CD123 CAR-T cells. The City of Hope Medical Center conducted a CD123 CAR-T cells trial, using a lentivirally-transduced second-generation CAR, with a dose-escalation (50.10^6 or 200.10^6 /kg) in 6 AML patients. Interim results reported transient cytological response for one of the 2 patients treated with the lower dose, and 2 of the 4 patients receiving the higher dose experienced complete remission and went on to allo-HSCT. The 2 others had transient partial response. No dose limiting toxicity, including cytopenia, was observed at the time of the report[48]. Finally, CLL-1, the C-type lectin molecule-1, is another interesting target due to its high expression in AML and absence in healthy HSPC. As more than 60% of AML who express CD33 also express CLL-1, dual-specific CAR-T cells were designed by Liu and colleagues from China and driven to first-in human trial. In a preliminary report, they describe two patients with R/R AML who experienced MRD-negative CR, accompanied by pancytopenia within 3 weeks after CAR-T cell infusion. These patients later underwent allo-HSCT with subsequent hematopoietic recovery[49].

CAR-T cells safety

These early clinical trials of CD19 CAR-T cells rapidly uncovered immune mediated toxicities, including CRS and immune effector cell-associated neurotoxicity syndrome (ICANS), but also “on-target, off-tumor” effect. The CRS is an immune-mediated cytokine storm resulting from intense antitumor response mediated by excessive activation and proliferation of CAR-T cells. Clinically, it encompasses several symptoms of systemic inflammation ranging from fever to rigors, hypotension, hypoxia, respiratory failure, acute kidney injury, coagulopathy and multiorgan dysfunction[50]. Along with CRS, another common toxicity observed after CAR-T cell therapy is neurotoxicity or ICANS. The mechanism is believed to be related to endothelial activation and blood-brain barrier disruption[51], and more recently it was demonstrated that mural cells, that surround the endothelium and are critical for blood-brain-barrier integrity, express CD19[52]. ICANS clinically manifests with the deterioration of neurological function starting from word-finding difficulty to a depressed level of consciousness, seizures, and rarely cerebral edema. Finally, “on-target, off-tumor” toxicity refers to immune-mediated destruction of normal tissues that express the targeted antigen. In the case of CD19-targeting T-cells, B-cell aplasia does not cause severe side effects. Based on the rituximab experience, hypogammaglobulinemia can be effectively managed by immunoglobulin supplementation for patients with recurrent infections.

Pivotal clinical trials leading to FDA approval

Driven by the success of early trials, phase II studies rapidly set up to validate the efficacy of CD19-targeted CAR-T cell therapy in patients with refractory hematological malignancies. Based on their successful results, three CD19 CAR-T cells products were approved in the United States and Europe as of December 2020.

B-cell lymphoma

The first multicenter phase II pivotal trial published was ZUMA-1, conducted in North America by Kite/Gilead, which corresponded to the continuation of the phase I trial conducted at the NCI cited above[53]. It evaluated axicabtagene ciloleucel (axi-cel, KTE-C19) in 111 patients with DLBCL, primary mediastinal B-cell lymphoma (PMBCL), or transformed FL with refractory disease. Patients received a dose of 2.10^6 anti-CD19 CAR-T cells/kg after a conditioning regimen of low-dose Cy/Flu (500 mg/m^2 and 30 mg/m^2 for 3 days). Of note, no bridging therapies were allowed between leukapheresis and Cy/Flu conditioning and median time from apheresis to CAR-T cells infusion was 24 days. Among the 111 patients who were enrolled, axi-cel was successfully administered to 101 (91%). The ORR was 82%, and the CR rate was 54%. With a median follow-up of 15.4 months, 42% of the patients continued to have a response, with 40% continuing to have a CR. CRS were observed in 93% of the patients, with 13% of grade 3 or higher and one lethal case, and neurological toxicity occurred in 64% of the patients and was severe in 28% of the cases. After a median follow-up of 15.4 months, median PFS was 5.8 months and median overall survival (OS) was not reach with an estimated 12-months OS of 59%. Based on this first successful clinical trial, axi-cel (YESCARTA®) was approved in October 2017 by the FDA and in June 2018 by the EMA for adults with relapsed or refractory DLBCL or PMBCL after 2 or more lines. Updated data from ZUMA-1 showed that Axi-cel elicits 47% 3-years OS.

The ZUMA-2 trial evaluated brexucabtagene autoleucel (KTE-X19), in 74 patients with R/R mantle-cell lymphoma (MCL)[54]. The primary efficacy analysis showed an ORR of 93% and CR rate of 63% in the 60 patients included in the primary efficacy analysis. At a median follow-up of 12.3 months, 57% of these patients were in remission. Common adverse events were cytopenias (94%) and infections (32%) with 2 infectious-related death and grade 3 or higher CRS and ICANS occurred in 15% and 31% of patients respectively. Results from ZUMA-2 indicate that MCL patient can

greatly benefit from CAR-T cells, although at the expense of a high risk of toxicities. In June 2020, the FDA approved brexucabtagene autoleucel (TECARTUS®) for the treatment of R/R MCL.

The second large international phase II clinical trial JULIET was conducted by Novartis in 111 patients with R/R DLBCL[55]. In this trial, 91% of the patients received a bridging therapy. Tisagenlecleucel (tisa-ce, CTL019) was administered following lymphodepleting regimen by Cy/Flu (lower dose, 250mg/m² and 25mg/m² respectively) or bendamustine, at variable dose ranging from 1.10⁷ to 6.10⁸ CAR-T cells. Among the 93 patients infused and included in the efficacy evaluation, ORR was 52% with 40% of CR rate. Response rates were consistent across prognostic subgroups. The most common grade 3 or 4 adverse events of special interest included CRS (22%), neurologic events (12%), cytopenias lasting more than 28 days (32%), infections (20%), and febrile neutropenia (14%). Updated analysis showed that with a median follow-up of 14 months, median PFS was 2.9 months and median OS was 12 months. Approval for tisa-cel (KYMRIA®) was consequently granted in May 2018 by the FDA and August 2018 by the EMA for adults with R/R DLBCL, high grade B-cell lymphoma and transformed FL after two or more lines. Updated analysis from the JULIET trial finds sustained response with 2-years OS of 40%.

TRANSCEND NHL-001 was the last large trial recently published and conducted by Juno therapeutics/Celgene/BMS[56]. In this trial, lisocabtagene maraleucel (liso-cel, JCAR017) was administered to R/R patient with DLBCL, high grade B-cell lymphoma, DLBCL transformed from any indolent lymphoma, PMBCL and FL grade 3B. Patients were assigned to one of three target dose levels: 50.10⁶ (one or two doses), 100.10⁶ and 150.10⁶ CAR-T cells, which were administered as a sequential infusion of two components (CD8+ and CD4+ CAR-T cells) at equal target doses. Of 256 patients included in the efficacy-evaluable set, an OR was achieved in 73% of the patients and a CR in 53%. CRS and neurological events occurred in 42% and 30% of the patients, respectively, being severe in 2% and 10% respectively. Overall safety and activity of liso-cel did not differ by dose level. Liso-cel (JCAR017) is currently being reviewed by the FDA for an approval.

The **Table 3** resume the 3 main studies of CD19 CAR-T cells conducted in R/R B-cell lymphomas.

B-cell acute lymphoblastic leukemia

The large clinical trial ELIANA was conducted between 2015 and 2017 by the CHOP's group in collaboration with Novartis[57]. In this trial, 75 children and young adults were treated with axicabtagene ciloleucel (axi-cel, KTE-C19). The overall remission rate within 3 months was 81% (61/75), with all patients who had a response to treatment found to be negative for MRD by flow cytometry. The rates of event-free survival (EFS) and OS were 73% and 90% respectively, at 6 months and 50% and 76% at 12 months. The median duration of remission was not reached. CRS occurred in 77% of patients, 46% being severe and neurologic events occurred in 40% of patients with 13% grade 3. Based on this trial, tisagenlecleucel (KYMRIA®) was FDA approved in August 2017 for patients up to 25 years with B-ALL who do not respond to treatment or have relapsed two or more times. EMA approval came later in June 2018.

Multiple myeloma

Based on preclinical studies in showing potent *in vitro* and *in vivo* killing of myeloma tumor cells by BCMA CAR-T cells, a phase I trial was conducted in collaboration with Bluebird bio with the BCMA-4-1BB CAR-T cells idecabtagene vicleucel (Ide-cel, bb2121)[58]. In this trial published in May 2019, 33 heavily pretreated patients with R/R MM received bb2121. The OR rate was 85%, including 15 patients (45%) with CR. The median PFS was 11.8 months. All 16 patients who had a response (partial response or better) and who could be evaluated for MRD had MRD-negative status. CAR-T cells persisted up to 1 year after the infusion. Hematologic toxic effects were the most common events, including neutropenia (85%), leukopenia (58%), anemia (45%), and thrombocytopenia (45%). A total of 25 patients (76%) had CRS, which was of grade 3 in 6%. Neurologic effects occurred in 14 patients (42%) and were of grade 3 or higher in 6%. Based on these encouraging results, Ide-cel (bb2121) is being FDA evaluated and approval is expected in March 2021.

Ongoing clinical trials

As of December 2020, there are more than 500 ongoing clinical trials worldwide involving CAR-T cells registered on the clinicaltrials.gov website, with more than half of them arising from China, approximately 40% in the United States and less than 10% in Europe[59]. In Europe, approximately sixty clinical trial are registered with the vast majority being conducted in hematological malignancies (i.e., DLBCL, CLL, low grade lymphoma, Hodgkin lymphoma) and less than 10% in solid tumors. In France, there are about ten clinical trials currently recruiting, most of them are international trials sponsored by pharmaceutical companies, and we can highlight two French academic trials. The first one is conducted by Dr Ana Berceanu and Dr Christophe Ferrand in Besançon and aim at evaluating interleukin 1 receptor accessory protein (IL1RAP) expression on AML in order to design specific CAR-T cells for IL1RAP-expressing AML. This team has long been interested in targeting IL1RAP through CAR-T cells, first in chronic myeloid leukemia

(CML) then in AML, with successful preclinical data. The second French initiative is the phase II trial ALYCANTE led by Pr Roch Houot with the Lymphoma Study Association (LYSA) group which evaluate Axi-cel as a second-line treatment in patients with R/R aggressive B lymphoma ineligible to ASCT. Lastly, Pr Nicolas Boissel at Saint Louis Hospital is setting up a multicenter phase I/IIa trial DualCALM01 to evaluate dual-CD19-CD22 CAR-T cells for R/R B-cell malignancies.

CONCLUSION

T cells genetically engineered to express CAR have emerged as revolutionary therapeutic tools in the field of B-cell hematological malignancies. It took many years of basic science discovery to generate the first CAR construct some 30 years ago and subsequent stepwise evolution in their development to enhance the power of this approach. Today, we are still at the very early steps of these adoptive cellular therapies and much remain to be learned and improved with endless engineering strategies. Even though proof of concept of the efficiency of CAR-T cells has been established only 10 years ago, subsequent trials of CD19-targeted CAR-T cell therapy have shown unparalleled survival outcomes in heavily pretreated patients with chemo refractory B-cell lymphoma or B-ALL, leading to FDA and EMA approval of three CAR products. Emerging real-world evidence seems to confirm these promising results. Therefore, there has been a paradigm shift in hematology with the establishment of immune cell-based therapies, including CAR-T cells, as powerful alternative for chemo refractory patients. The development of off-the-shelf universal CAR-T cells readily available at a reduced cost remains among the top priority. Finally, one major future challenge is to translate this success to other subtypes of hematological malignancies but also to solid tumors where initial attempts have been largely ineffective, still they account for the majority of cancer-related death.

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FIGURES and LEGENDS

Figure 1. Milestones in the CAR-T cells development.

This timeline shows major breakthrough in CAR-T cell development, from early CAR design to first clinical application (A) and most recent successful pivotal trials leading to FDA approval (B).

B-ALL: B-cell acute lymphoblastic leukemia, BCMA: B-cell maturation antigen, CAR : Chimeric Antigen Receptor, CAIX : Carbonic Anhydrase IX, CLL : chronic lymphocytic leukemia, DLBCL: diffuse large B-cell lymphoma, EMA: European Medicines Agency, FDA: Food and Drug Administration, tFL: transformed follicular lymphoma, FR: folate receptor, scFv : single chain Fragment variable, HGBCL: high grade B-cell lymphoma, Ig-TCR: Immunoglobulin-T-Cell Receptor, MCL: mantle cell lymphoma, NHL: non Hodgkin lymphoma, NK: natural killer, PMBCL: primary mediastinal B-cell lymphoma, R/R: relapsed/Refractory, TRAC: T-cell receptor alpha chain, TRUCK: T-cells redirected for antigen-unrestricted cytokine-mediated killing

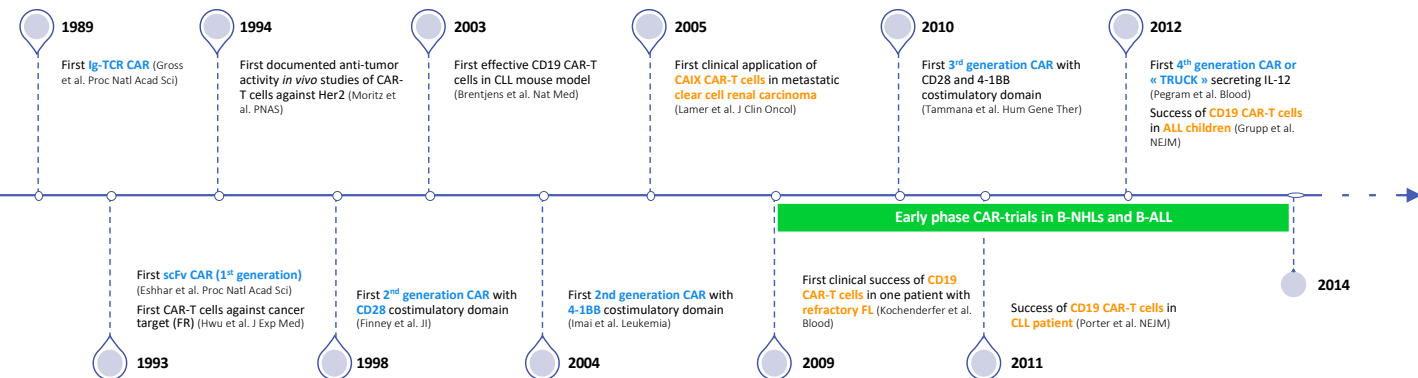
Figure 2. From T-cell receptor to chimeric antigen receptor.

Left, B-cell receptor (BCR) are composed of an immunoglobulin forming a transmembrane receptor protein and T-cell receptor (TCR) is composed of an antigen-recognizing unit, $\alpha\beta$ or $\gamma\delta$ heterodimer and a signaling transduction ensemble, the CD complex ($\delta\epsilon\text{-}\gamma\epsilon\text{-}\zeta\zeta$). Right, T-bodies are T cells redirected to specific tumors antigen by the introduction of transgenic TCRs (“double chain-TCR”) or CAR (chimeric antigen receptor) proteins. CARs are fusion proteins composed of an extracellular portion that is derived from an antibody (scFv) and intracellular signaling modules derived from T-cell signaling proteins. First-generation CARs contain CD3 ζ , whereas second-generation CARs possess a costimulatory endodomain (e.g., CD28 or 4-1BB) fused to CD3 ζ . Third-generation CARs consist of two costimulatory domains linked to CD3 ζ . Fourth-generation CARs include cytokine secretion or additional costimulatory.

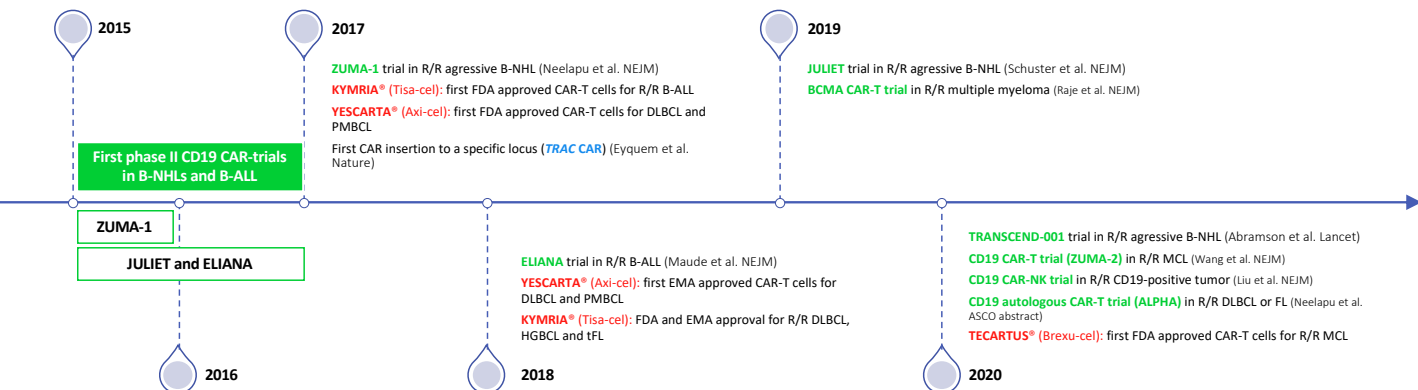
CAR: Chimeric Antigen Receptor, CH: constant heavy, CL: constant light, CM: costimulatory molecule, IL: interleukin, ITAM: Immunoreceptor Tyrosine-based Activating Motifs, scFv: single chain Fragment variable, TCR: T-Cell Receptor, VH: variable heavy, VL: variable light.

Figure 1.

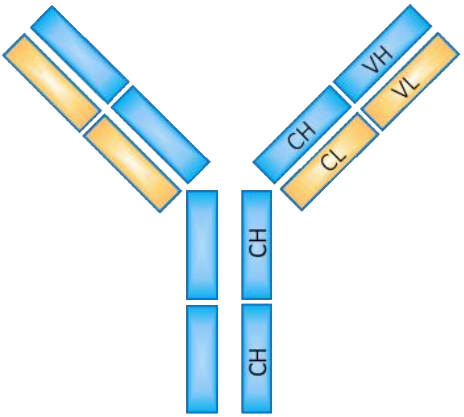
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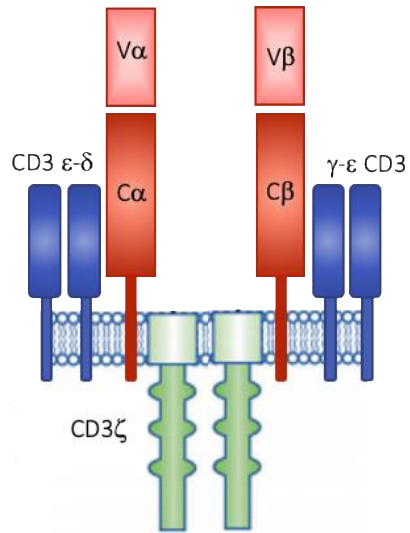
B



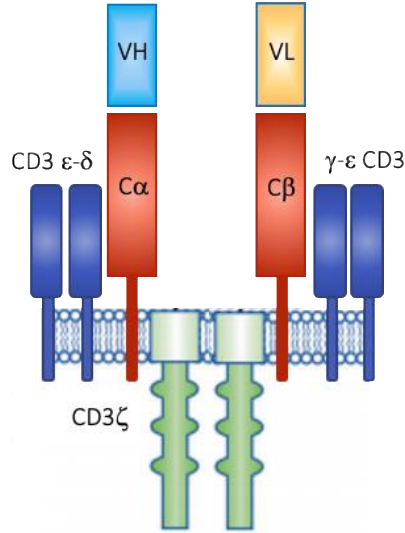
Immunoglobulin



TCR



Double chain chimeric receptor



T-Bodies

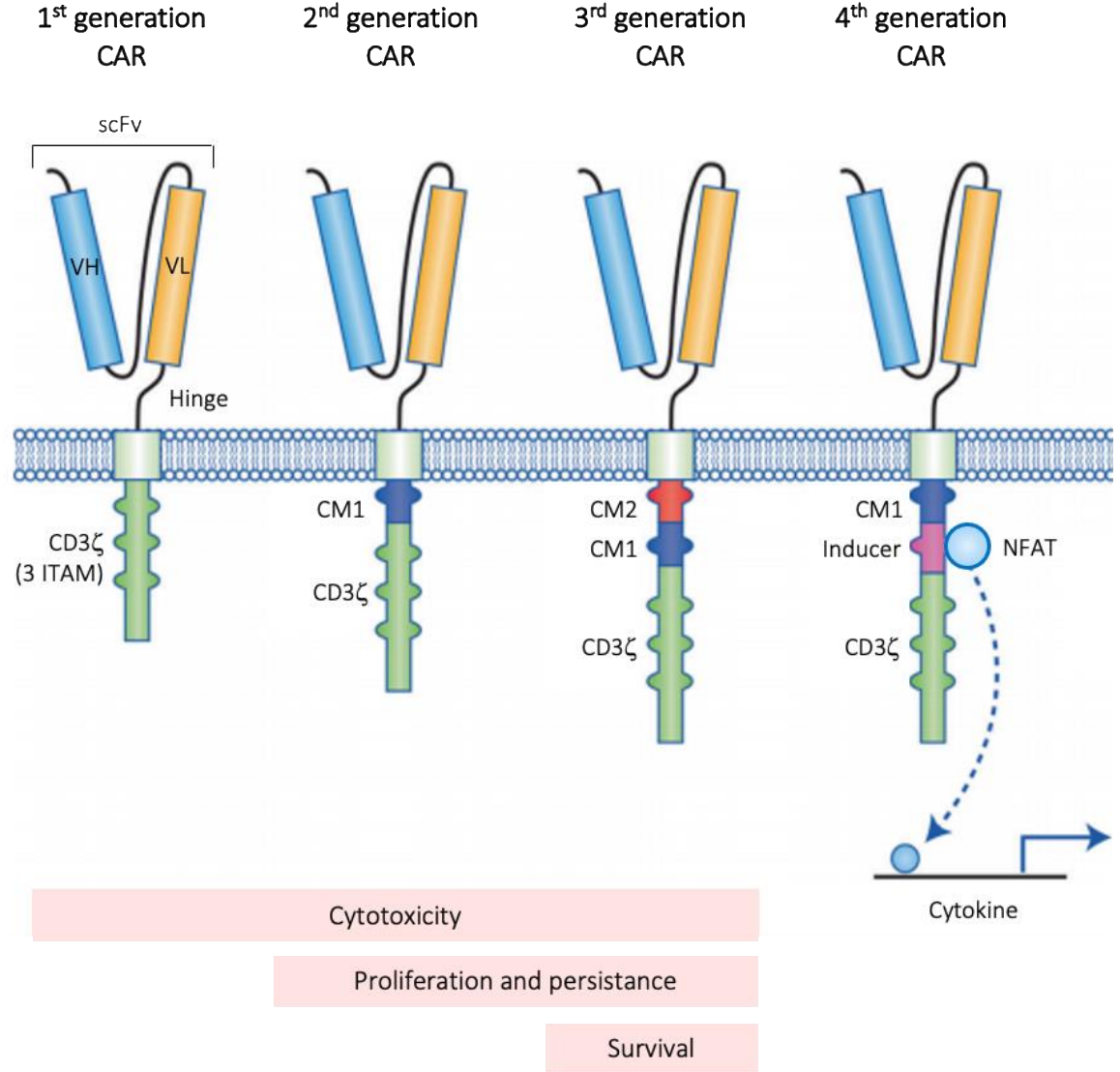


Table 1. Early clinical trials for CAR-T cells in B-cell lymphomas.

Institution and authors	CAR construct	Viral vector	Dose (T-cells/kg)	Patients population	ORR	CRS/sCRS	ICANS/sICANS	Long term results
National Cancer institute								
Kochenderfer et al. JCO, 2015 and Mol Ther, 2017	CD19-CD28 ζ	Retrovirus	Reduction from 5 to 1.10 ⁶ (toxicity)	15 (9 DLBCL and 6 indolent NHLs)	92% (62% CR)	87%/NA	33%/NA	4/9 patient with persistent CR (38 to 56 months)
University of Pennsylvania								
Schuster et al. NEJM, 2017	CD19-4-1BB ζ	Lentivirus	1 to 5.10 ⁸	28 (14 DLBCL and 14 FL)	43% DLBCL 71% FL	57%/18%	39%/11%	6/14 DLBCL and 8/14 FL with persistent CR (follow up 8 to 37 months)
Fred Hutchinson Cancer Research Center								
Turtle et al. Sci Trans Med, 2016	CD19-4-1BB ζ CD4/CD8 1:1	Lentivirus	Reduction from 2.10 ⁷ to 2.10 ⁶ (toxicity)	32 (11 DLBCL, 11 TFL, 6 FL and 4 MCL)	64% DLBCL 70% TFLBCL 80% FL 25% MCL	63%/28%	0/25%	NA

CAR: Chimeric Antigen Receptor, CR: Complete Response, CRS: Cytokine Release Syndrome, sCRS: severe CRS (grade \geq 3), Cy: Cyclophosphamide, DLBCL: Diffuse Large B-Cell Lymphoma, FL: Follicular Lymphoma, ICANS: Immune Cell Associated Neurotoxicity Syndrome, sICANS: severe ICANS (grade \geq 3), MCL: Mantle Cell Lymphoma, NHLs: Non-Hodgkin Lymphomas, ORR: Overall Response Rate, OS: Overall Survival, TFL: Transformed Follicular Lymphoma.
 NA: Not Assessed

Table 2. Early clinical trials for CAR-T cells in B-ALL.

Institution and authors	CAR construct	Viral vector	Dose (T-cells/kg)	Patient population	CR	CRS/sCRS	ICANS/sICANS	Long term results
University of Pennsylvania								
Maude et al. NEJM, 2014	CD19-4-1BB ζ	Lentiviral	0.76 to 20.6 $\times 10^6$	30 Children + YA	mCR=90% MRD=73%	100%/27%	43%	6-months OS=78%
National Cancer Institute								
Lee et al. Blood abstract, 2016	CD19-CD28 ζ	Retroviral	1 or 3.10 ⁶	51 Children + YA	mCR=61% MRD=55%	NA/14%	NA/10%	mLFS in MRD- =18 months
Fred Hutchinson Cancer Research Center								
Turtle et al. J Clin Invest, 2016	CD19-4-1BB ζ CD4/CD8 1:1	Lentiviral	2.10 ⁵ , 2.10 ⁶ , or 2.10 ⁷	30 Adults	MRD=93%	83%/23%	50%/50%	NA
Gardner et al. Blood, 2017	CD19-4-1BB ζ CD4/CD8 1:1	Lentiviral	0.5, 1, 5 or 10.10 ⁶	43 Children + YA	MRD=93%	90%/23%	49%/21%	12-months OS=69.5%
Memorial Sloan Kettering Cancer Center								
Park et al. NEJM, 2018	CD19-CD28 ζ	Retroviral	1 or 3.10 ⁶	53 Adults	mCR=83% MRD=67%	85%/26%	46%/42%	Median OS=12.9 months

CAR: Chimeric Antigen Receptor, CR: Complete Response, mCR: morphological CR, CRS: Cytokine Release Syndrome, sCRS: severe CRS, MRD: Minimal Residual Disease assessed by flow cytometry, ICANS: Immune Cell Associated Neurotoxicity Syndrome, sICANS: severe ICANS, mLFS: morphological Leukemia Free State, NA: Not Assessed, ORR: Overall Response Rate, OS: Overall Survival, YA: Young Adult.
NA: Not Assessed

Table 3. Summary of phase II pivotal clinical trials for CAR-T cells in B-cell lymphoma.

Trial	ZUMA-1	JULIET	TRANSCEND NHL-001	ZUMA-2
Publication	Neelapu et al. NEJM, 2017	Schuster et al. NEJM, 2019	Abramson et al. The Lancet, 2020	Wang et al. NEJM, 2020
Institution	MDACC	UPenn	Fred Hutch	MDACC
Firm	Kite/Gilead	Novartis	Juno/Celgene	Kite/Gilead
Years	2015-2016	2015-2017	2016-2019	2016-2019
CAR T-cells	KTE-C19 Axicabtagene ciloleucel (Axi-cel)	CTL019 Tisagenlecleucel (Tisa-cel)	JCAR17 Lisocabtagene maraleucel (Liso-cel)	KTE-X19 Brexucabtagene autoleucel (Brexu-cel)
CAR construct	CD19-CD28 ζ CAR	CD19-4-1BB ζ CAR	CD19-4-1BB ζ CAR CD4/CD8 1:1	CD19-CD28 ζ CAR
Viral vector	Retrovirus	Lentivirus	Lentivirus	Retrovirus
Bridging therapy	0	92%	59%	37%
Dose (T-cells/kg)	2.10 ⁶	0.1 to 6.10 ⁸	50, 100 or 150.10 ⁶	2.10 ⁶
Lymphodepleting chemotherapy	Cy (500 mg/m ²)/Flu (30 mg/m ²) x 3 days	Cy (250 mg/m ²)/Flu (25 mg/m ²) or Bendamustine x 3 days	Cy (300 mg/m ²)/Flu (30 mg/m ²) x 3 days	Cy (500 mg/m ²)/Flu (30 mg/m ²) x 3 days
Patient included infused analyzed for efficacy	111 Phase I: 7, Phase II: 101 108	165 111 93	344 269 256	74 68 60
Histology	77 (76%) DLBCL 16 (16%) TFL 8 (8%) PMBCL	88 (79%) DLBCL, NOS 21 (19%) TFL 2 (2%) others	137 (51%) DLBCL 78 (29%) DLBCLt 36 (13%) high grade DLBCL 15 (6%) PMBCL 3 (1%) Grade 3B FL	74 (100%) MCL
Median age (years)	58	56	63	65
ECOG PS 0/1/2	42(42%)/59(58%)/0	61(55%)/50(45%)/0	110 (41%)/155(58%)/4(1%)	44 (65%)/24 (35%)/0
Ann Arbor III-IV	86 (85%)	84 (76%)	NA	58 (85%) stage IV
\geq 3 prior therapy	70 (69%)	57 (52%)	139 (51%)	55 (81%)
Median follow up (months)	15.4	14	18.8	12.3
ORR	82%	52%	73%	93%
CR	54%	40%	53%	67%
CR at 6 months	~40%	30%	41%	NA
OS at 12 months	60%	49%	58%	83%
CRS grade I-II/III-IV	81(80%)/13(13%)	40(36%)/24(22%)	107(40%)/6(2%)	52 (76%)/10 (15%)
ICANS grade I-II/III-IV	37(37%)/28(28%)	10(9%)/13(12%)	53(20%)/27(10%)	22 (32%)/21 (31%)

CAR: Chimeric Antigen Receptor, CR: Complete Response, CRS: Cytokine Release Syndrome, Cy: Cyclophosphamide, DLBCL: Diffuse Large B-Cell Lymphoma, DLBCLt: DLBCL transformed from indolent lymphoma, ECOG: Eastern Cooperative Oncology Group, Flu: Fludarabine, ICANS: Immune Cell Associated Neurotoxicity Syndrome, IPI: International Prognostic Scoring, MCL: mantle cell lymphoma, NOS: Not Otherwise Specified, PMBCL: Primary Mediastinal B-Cell Lymphoma, ORR: Overall Response Rate, OS: Overall Survival, PS: Performance Status, TFL: Transformed Follicular Lymphoma.
NA: Not Assessed