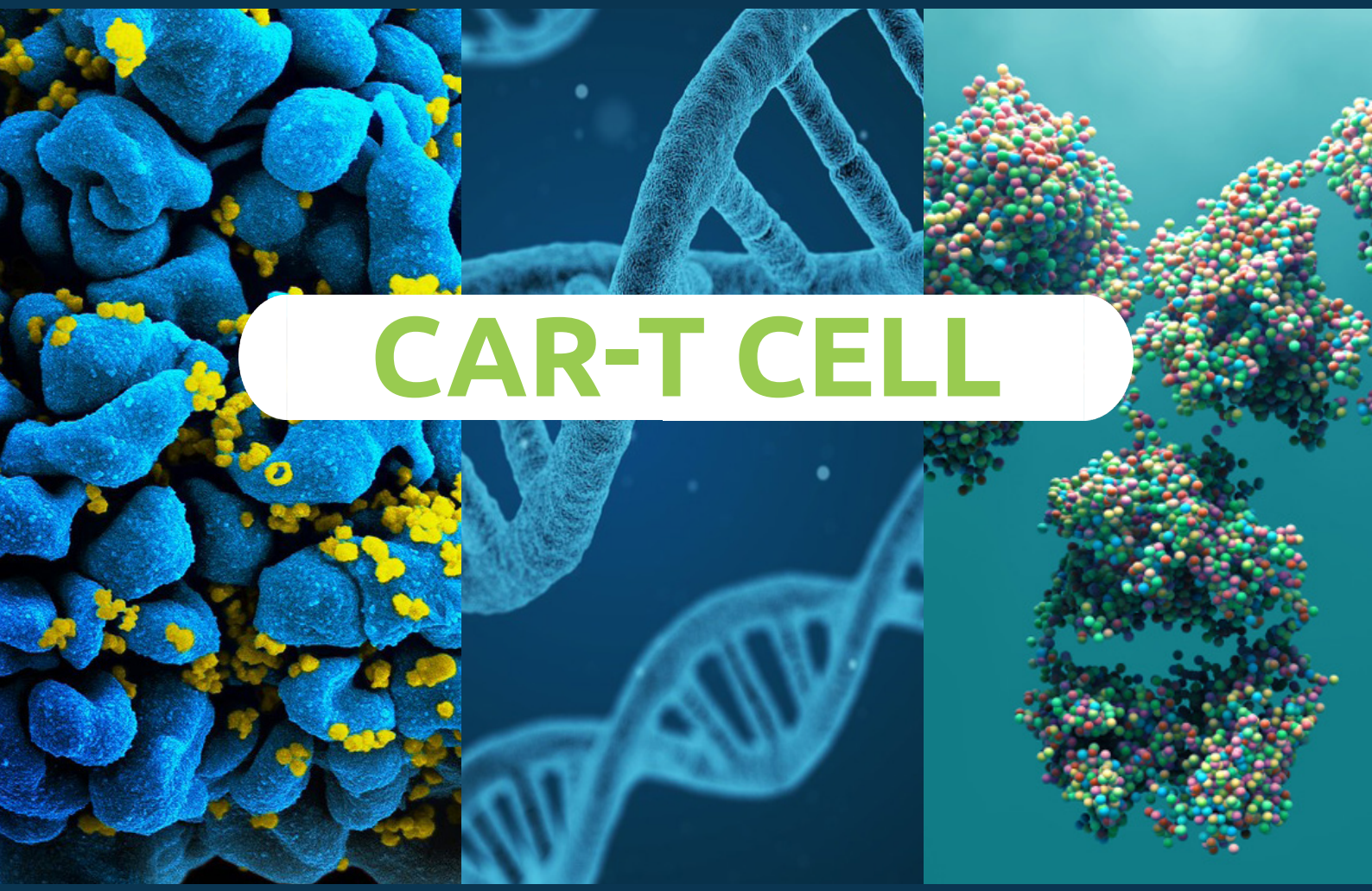
IMMUNOWATCH

EDITION N°2 – NOVEMBER 2020



CAR-T CELL

MABDESIGN
THE IMMUNOTHERAPY NETWORK

INTRODUCTION

MabDesign's Immunowatch is a one-of-a-kind information monitoring newsletter in the field of immunotherapy. Its aim is to provide members of our association with the most recent and pertinent data gathered or generated through the key expertise of MabDesign and its collaborators in scientific research, business intelligence, market analysis and intellectual property.

Each edition will focus on one trending type of immunotherapy. It's general format will include a market study research, a selection of scientific publications, financial and economic information, a special opinion article and a section dedicated to intellectual property. The content of each edition will be decided by an editorial composed of two field experts, one from academia and one from the industry. Immunowatch is done in collaboration with the MAbMapping Unit of the Ambition Recherche & Développement (ARD) Biomédicaments 2020 Phase II programme, funded by the Centre Val de Loire region.

BIOPHARMACEUTICALS
Innovation Dynamics in Health
IN REGION CENTRE-VAL DE LOIRE

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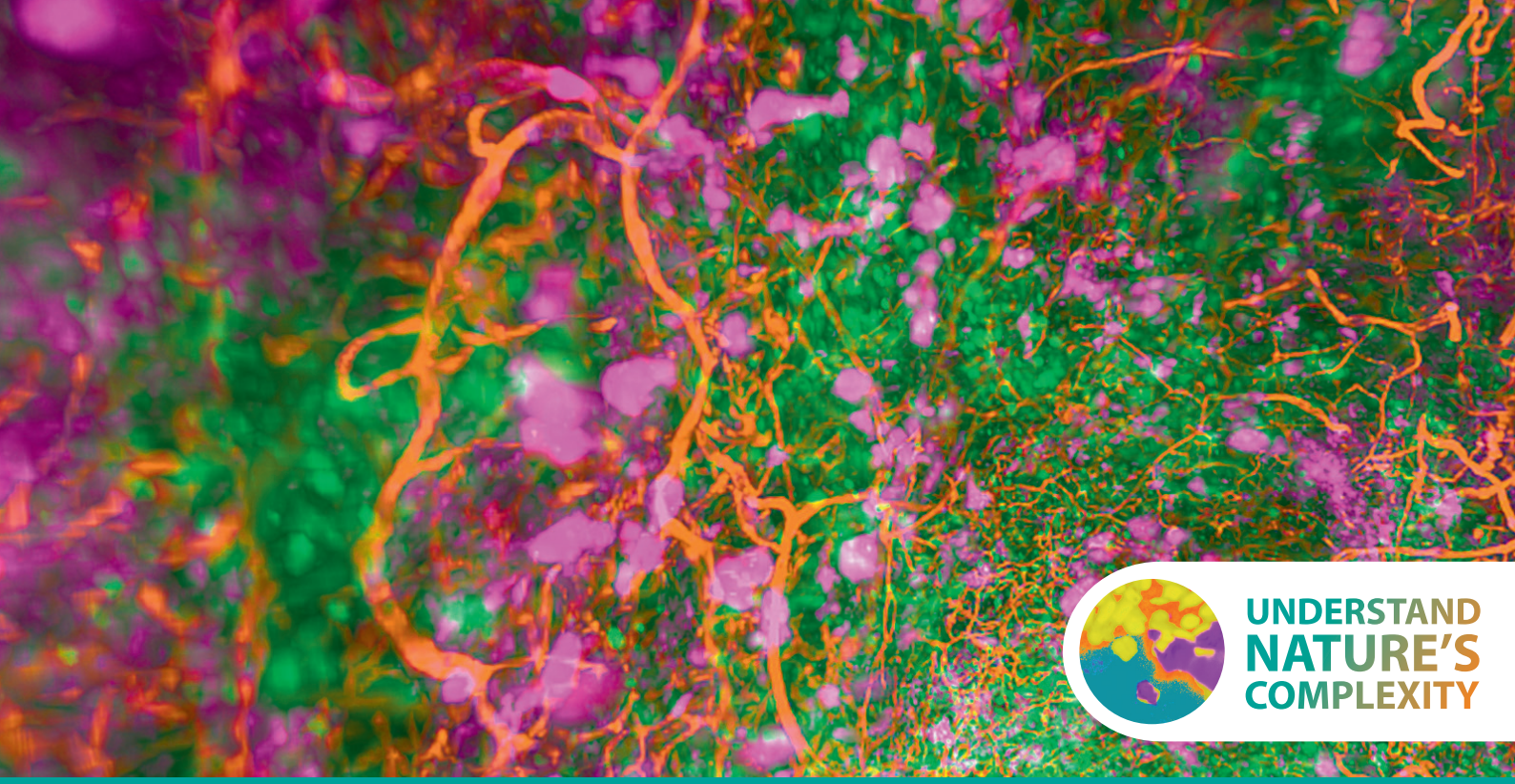

Visualize cancer & immune cells

CAR T cells (in pink) from pancreatic carcinoma (in green).

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EDITORIAL

Jérôme Larghero

Jerome Larghero is professor of cell biology at the Paris University School of Medicine, and Director of the Department of Biotherapies at Saint-Louis Hospital, AP-HP, Paris. He heads the Saint-Louis Hospital Cell Therapy Unit and the AP-HP Cord Blood Bank, and coordinates the Clinical Investigation Center in Biotherapies. He also directed the AP-HP MEARY Cell and Gene Therapy Center, dedicated to the manufacturing of advanced therapies medicinal products. He is involved in CAR-T cells therapy programs since end 2016. He conducted his research activities in the team "Stem Cells Biotechnologies" he directed at Inserm Unit U976. He is the former Director of the "Hematology-Oncogenesis-Biotherapies" Doctoral School.
Jerome Larghero is a member of the French Academy of Medicine and of the French Academy of Pharmacy, vice-president of the commission "Biothérapies & Molécules" of the Medicen Healthtech Innovation Hub, member of the guidance council of the Agence de la Biomédecine. He has been awarded by the great price of the Academy of Science for cell therapy and regenerative medicine in 2014 and by the Galien price in 2016.

Ibtissam Marchiq

Ibtissam Marchiq, , Ph.D, joined SERVIER IDRS early 2016, first as junior scientist and then as Project Leader in the Immuno-Oncology Research Program. She received her graduate degree in Molecular Biology and Cancer Metabolism from the University of Nice-Sophia Antipolis. During her PhD, she developed strong expertise in gene editing technologies and tumor-stroma interactions. At SERVIER, she consolidated her knowledge on Immuno-Oncology and T cell biology by working on allogeneic CAR T cell therapy for hematological malignancies and solid tumors. Today, she is leading innovative preclinical research programs aiming to improve the characteristics of allogeneic CAR T cells to increase their efficacy with lowering their toxicity. She is also involved in the T2Evolve -IMI program aiming to accelerate the development and improving the access to CAR and TCR engineered T cell therapy.

Hélène Negre

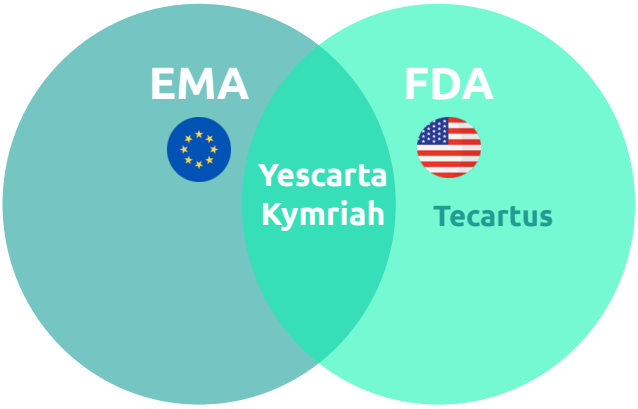
Helene NEGRE, PharmD, phD, developed her expertise in Cell & Gene Therapy CMC through more than 20 years of experience in Advanced Therapy Medicinal Product (ATMPs). Initially, she worked at Pitié-Salpêtrière Hospital (AP-HP) in the Biotherapy department (Pr. Klatzmann) in charge of hematopoietic stem cell transplant processing. In 2014, she joined Dana Farber Cancer Institute (Boston) and worked for 5 years as technical Director of the Novel Cell therapy team in the Cell Manipulation Core Facility (Pr. Ritz). This unit develops novel cell manufacturing procedures and manufactures a variety of Cell and Gene Drug products: CAR-T cells, GMP IPS cell lines, CD34+ transduced cells, Mesenchymal Stromal Cells, Treg, NK cells… Since September 2019, she is CMC Project leader for the allogeneic CAR T program at SERVIER IRIS and EPFIA project leader for a new IMI program T2Evolve on autologous and allogeneic engineered T cells.

GLOBAL CAR-T CELL market

Discover the available products, pipeline candidates, major deals and CAR-T companies

CAR-T cell market in 2020*

List of currently approved CAR-T Cell



Drug name	Brand name	Company	Indication	Target	Vector	Manufacturing sites in EU (In-House)
axicabtagene ciloleucel	Yescarta	Gilead / Kite	Diffuse Large B-Cell Lymphoma	CD19	Retrovirus	Amsterdam (Nether-lands)
tisagenlecleucel	Kymriah	Novartis	B-Cell Acute Lymphocytic Leukemia	CD19	Lentivirus	Les Ulis (France) Stein (Suisse)
brexucabtagene autoleucel	Tecartus	Gilead / Kite	Mantle Cell Lymphoma	CD19	Retrovirus	NA

Development assistance of regulatory authorities

CAR T : Regulatory designation type	
Accelerated assessment	Breakthrough Therapy
PRIME Designation	Priority Review
Orphan Drug designation	Orphan Drug designation

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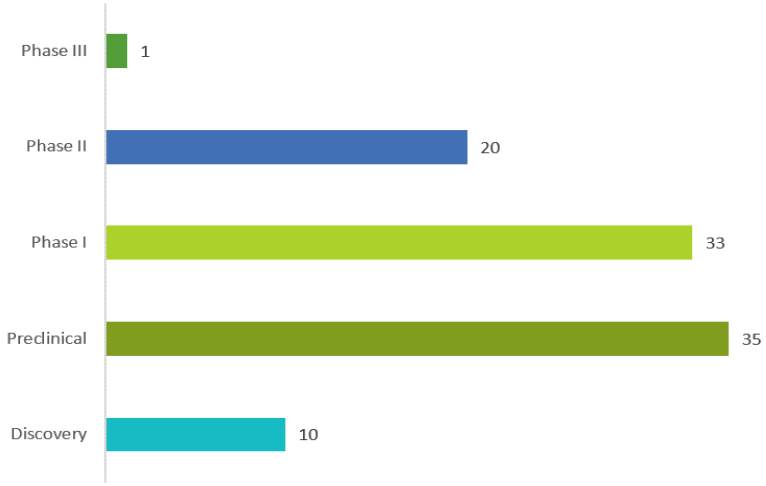
Source: Globaldata

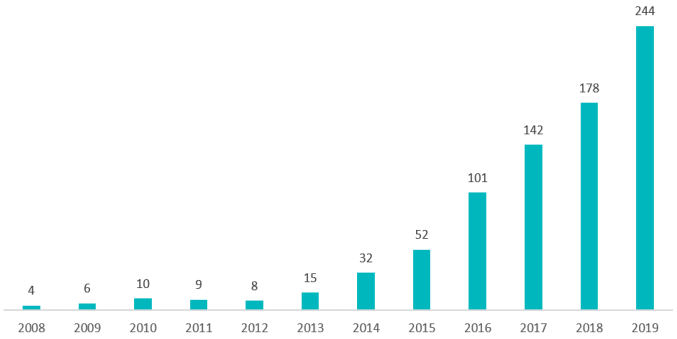
CAR-T cell pipeline in 2020*

Evolution of number of clinical trials

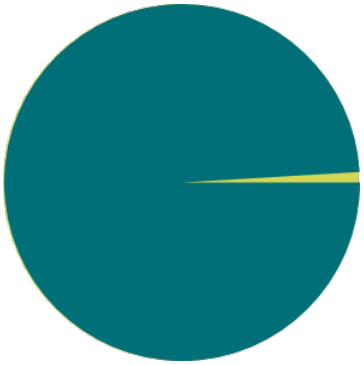
No. of CAR-T cell products under development



Evolution of number of clinical trials



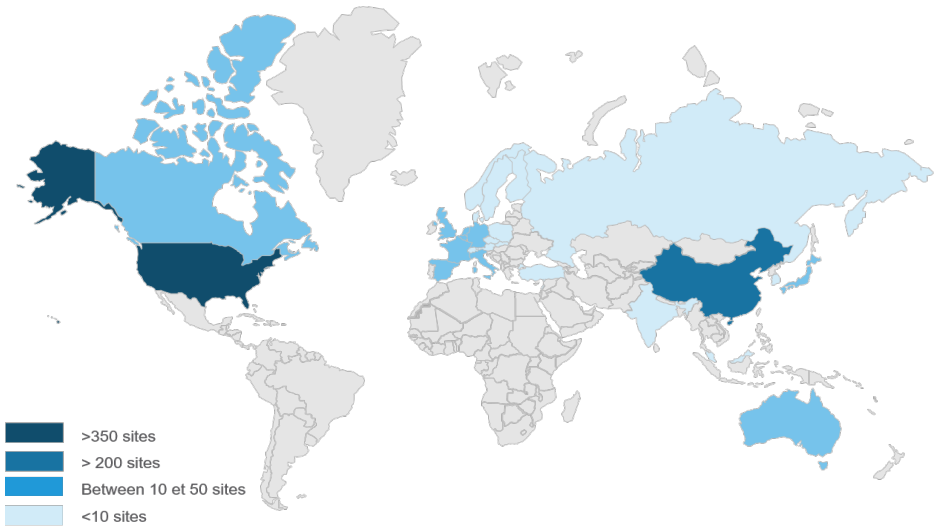
Proportion of clinical trials in therapy areas



other : 1.5 %

oncology : 98.5 %

Clinical trials by site location



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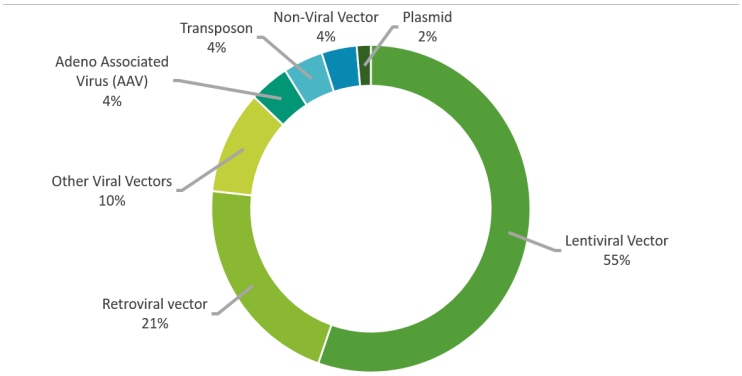
CAR-T cell pipeline in 2020*

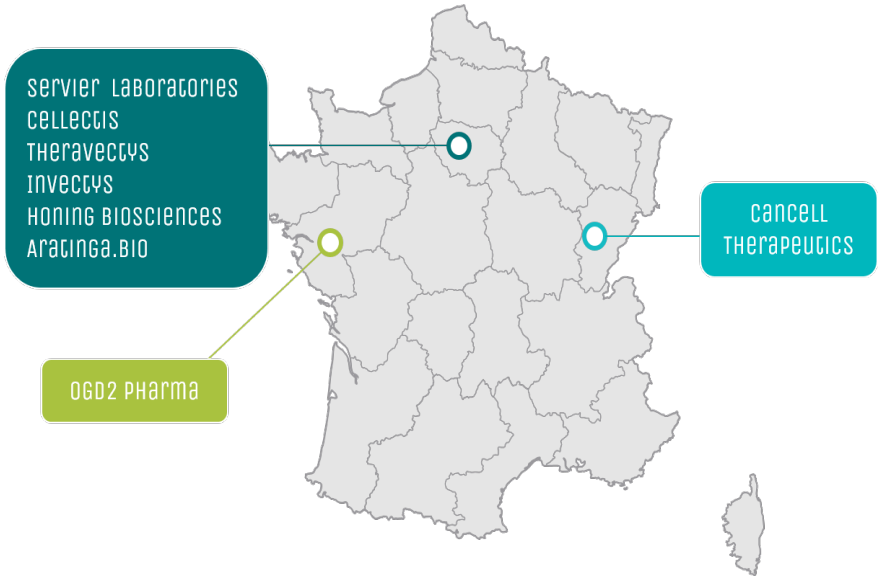
Types of vectors used



Top 3 targets

Top 5 companies with the most ongoing trials

CAR-T cell companies in France



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CAR-T cell : deals and companies

Summary of deals in the CAR-T cell field since 2000

Acquisition	Strategic Alliances	Capital Raisings
114 BILLION US$	22 BILLION US$	22 BILLION US$

TOP 3 deals across each category

Acquisition

Acquirers	Issuer	Deal value
Bristol-Myers Squibb	Celgene	89 (billion US$)
Gilead Sciences	Kite Pharma	12 (billion US$)
Celgene	Juno Therapeutic	9 (billion US$)

Capital Raising

Acquirers	Deal value
Bluebird bio	651 (million US$)
Bluebird bio	632 (million US$)
Allogene Therapeutics	550 (million US$)

Strategic Alliances

Partners		Deal value	Deals description
Fate Therapeutic	Janssen Biotech	3.1 (billion US$)	Fate Therapeutics Enters into Co-Development Agreement with Janssen Biotech
Cellectis	Pfizer	2.9 (billion US$)	Pfizer Enters into Licensing Agreement with Cellectis
Cellectis	Allogene Therapeutics	2.9 (billion US$)	Allogene Therapeutics Enters into Licensing Agreement with Cellectis

** All data has been generated by MabDesign unless stated otherwise*
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Special articles

Read the different inputs from the scientific community on various aspects of CAR-T

CAR-T CELLS IN B CELL ACUTE LYMPHOBLASTIC LEUKEMIA: STATE OF ART

Florence Rabian[1,2] and Nicolas Boissel[1,2]

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INTRODUCTION

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is a hematopoietic malignancy involving B-cell bone marrow progenitors. BCP-ALL is the most frequent cancer in children, with half of all cases diagnosed in this young population. Despite improving results after front-line chemotherapy, about 20-30% of patients still relapse with a very poor subsequent outcome. Immunotherapy including bispecific T-cell engager (blinatumomab) and CAR-T cells have recently enriched the armamentarium in BCP-ALL, especially in relapse/refractory (R/R) setting. Different CAR-T cell strategies have been developed with the aim to achieve durable response with a reasonable toxicity profile.

FIRST CAR-T CELLS DEVELOPMENT

The most advanced CAR-T cells target CD19 antigen expressed in the vast majority of BCP-ALL. Two autologous CD19 CAR-T cells (Auto-CART) are currently available to treat children and adults with R/R BCP-ALL, both containing CD3ζ activation domain but with different co-stimulation domain (4-1BB or CD28) for expansion and persistence.
Tisagenlecleucel (CTL019, 4-1BB costimulation) is approved in the US and in Europe for patients < 25 years old with R/R BCP-ALL (refractory, second relapse or first relapse after bone marrow stem cells transplantation). Axicabtagene ciloleucel (KTE-X19, CD28 costimulation) is currently developed in older patients (> 25 years old) and is available in this population under a nominative temporary use authorization.
Allogeneic strategies (alloCAR-T), also called universal CAR-T cells, are also investigated with the aim to reduce the time to access and to minimize manufacturing prices. The most advanced alloCAR-T is UCART19 that combines an anti-CD19/4-1BB/CD3ζ CAR expression, a deletion of the T-cell receptor alpha chain gene to prevent graft versus host disease, a deletion of CD52 gene to avoid sensitivity to alemtuzumab (anti-CD52) used in lymphodepletion regimen, and the expression of a suicide gene (CD20 mimotope) (1).

CAR-T CELLS RESULTS

Phase I/II studies for autologous CAR-T show complete remission (CR) rates ranging from 60 to 90% in advanced phase diseases, in which chemotherapy alone is expected to provide less than 40% CR rates (Table1). Almost all patients in complete remission also reach a complete minimal residual disease (MRD) response, meaning that the leukemic cells are not detectable by the most sensible techniques (10-4 sensitivity).
In the pivotal study ELIANA of CTL019 (2) and in the Seattle experience (3) with anti-CD19/4-1BB CAR-T, patient median age was respectively 11 years and 40 years old (ranges : 3-23 and 20-73 respectively), CR was achieved in 81% and 90% of patients infused respectively, and 1-year overall survival (OS) was 76% and 66% respectively. In the global trial ZUMA-3 of KTE-X19 (4) and in the MSKCC experience with anti-CD19/CD28 CAR-T (5,6) in adult patients, reported CR rates were 82%

with a median OS of 12.9 months for the MSKCC.
Relapse rates are around 30 to 50% in these different studies, which raises the question of the potential benefit of additional allogeneic hematopoietic stem cell transplantation (HSCT) after CAR-T cells. In BCP-ALL, relapses are observed in two main situations: 1) the lack of CAR-T persistence associated with a persistence of CD19 expression, 2) the loss of CD19 target, which may occur while CAR-T are persisting. Many mechanisms can lead to the loss of CD19 including CD19 gene deletion or mutation leading to alternative splicing and loss of specific epitope(7), leukemic lineage switch from B-ALL to acute myeloid leukemia, or defect in CD19 trafficking associated with intracytoplasmic CD19 retention.
CAR-T cells safety profile is influenced by many factors including patient age and condition, CAR construct and CAR-T infused dose, lymphodepletion, but also tumor burden before lymphodepletion (8). Like in other diseases, the two main early specific toxicities are cytokine release syndrome (CRS) and neurotoxicity (or ICANS, immune effector cell-associated neurotoxicity syndrome) (9). The CRS is a systemic inflammatory response syndrome due to cytokine release after CAR-T cells activation and expansion observed in 30-94% of patients. Neurotoxicity is reported in 20-64 % of patients in various forms (headaches, confusion, seizures…) and can occurs at the time of CRS or independently. Severe neurotoxicity is more frequent with CD28 than 4-1BB costimulation, reported in 63% and 42% of cases respectively after KTE-X19 or in the MSKCC experience (6), compared to 13% and 3% respectively in CTL019 studies (Table 1). These side effects had already been described after blinatumomab at lower frequency (10). Both can be life threatening but are mostly reversible with anti IL6 or IL6-R targeted therapy for CRS and steroids for neurotoxicity (11). Infectious complications are the most frequent long-term toxicity due to persistent B-cell aplasia associated with hypogammaglobulinemia. Intravenous immunoglobulin replacement may be required especially after long persisting CAR-T (4-1BB).

PERSPECTIVES

Future efforts focus on understanding the factors influencing CAR-T cells expansion, persistence, and response, which is essential to improve patient management while maintaining a good safety profile. Lymphodepletion regimen have already been improved these past years (12) with the generalization of fludarabine and cyclophosphamide combination (13) to improve CAR-T cells expansion and persistence but also the outcome of BCP-ALL and lymphoma patients. New CAR-T construct are intensively explored including other costimulation domains, receptor humanization, or improved killing capacities as in TRUCKS (T cells redirected for antigen-unrestricted cytokine-initiated killing) which combines cellular antitumor attack and immunomodulation by cytokine expression. New cell sources (i.e. NK/NKT cells…), control of CD4/CD8 ratio (14), reduced manufacturing time, or combination with immunomodulating agents including checkpoint inhibitors are also investigated. In BCP-ALL, one of the main goals remains to counter escape mechanisms. Many early phase trials have now reported on targeting new B-cell antigens including CD22, which in turn may be downregulated to induce tumor escape. Promising developments in the field include the sequential or dual targeting of two antigens (CD19, CD22) by combined or subsequent infusion of CD19 and CD22 CAR-Ts, or by the use of bispecific CD19/CD22 CAR-Ts. Finally, the place of CAR-T in frontline setting is also evaluated with the aim to improve the outcome of patients while limiting the toxicity of standard therapy combining high-dose chemotherapy and HSCT.

CONCLUSION

CAR-T cells is a highly promising therapy in R/R BCP-ALL. Future developments aim to increase CAR-T persistence, to improve safety profile, and to limit resistance and tumor escape.

Institution/ Protocol	NCT#	Tar-get	Costim.	Median age years (range)	Pts infused/ Pts enrolled	CRS Any grade/ Severe	Neuro-toxicity Any grade/ Severe	CR/ MRD-	Relapse	OS	LFS
CHOP	NCT01626495	CD19	4-1BB	11 (4-24)	59/–	52 (88) / 16 (27)	33 (85) / 2 of 25 (8)	52 (88%)	20 (34%)	79% at 12mo	55% at 12mo
SEATTLE	NCT02028455	CD19	4-1BB	12 (1-25)	43/45	40 (93) / 10 (23)	21 (49) / 9 (21)	40 (93%)	18 (45%)	66% at 12mo	51% at 12mo
ENSIGN	NCT02228096	CD19	4-1BB	12 (3-25)	29/35	26 (90) / 11 (38)	9 (31) / 1 (3)	18 (62%)	8 (44%)	76% at 6mo	66% at 6mo
ELIANA	NCT02228096	CD19	4-1BB	11 (3-23)	75/92	58 (77) / 35 (47)	30 (40) / 10 (13)	61 (81%)	17 (28%)	76% at 12mo	50% at 12mo
FHRCC	NCT01865617	CD19	4-1BB	40 (20-73)	30/32	25 (83) / 7 (23)	15 (50) / 15 (50)	27 (90%)	9 (33%)	-	-
MSKCC	NCT01044069	CD19	CD28	Adult	53/83	45 (85) / 14 (26)	23 (43) / 22 (42)	32 (62%)	16 (50%)	Median 12.9mo	median 6.1mo
ZUMA-3	NCT02614066	CD19	CD28	≥18	16/22	– / 4 (25)	– / 10 (63)	9/11 (82%)	4 (29%)	-	-
POB/NCI	NCT01593696	CD19	CD28	13 (3-30)	50/51	(76) / (13,5)	(25) / (6)	28 (56%)	8 (29%)	-	49.5% at 18mo**
POB/NCI	NCT02315612	CD22	4-1BB	19 (7-30)	21/22	16 (76.2) / 0	6 (37.5) / 0	9 (43%)	6 (66%)	-	-

Table 1. Summary of efficacy and safety results from selected clinical Trials of CAR-T cells in BCP-ALL

MRD–, minimal residual disease negative by flow cytometry; CR, complete remission; mo, months; MSKCC, Memorial Sloan Kettering Cancer Center; CHOP, Children's Hospital of Philadelphia; POB/NCI, Pediatric Oncology Branch/National Cancer Institute; FHCRC, Fred Hutchinson Cancer Research Center **LFS only given for complete responders

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Pharmaceutical and hospital circuits of CAR-T cells in a French hospital

Miryam Mebarki[1], Isabelle Madelaine[2]

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The arrival of CAR-T cells drug at the hospital required a complete reorganization of the pharmaceutical circuit of these innovative medicines. Health professionals from several departments are involved including clinical department, apheresis unit, cell therapy unit (CTU), hospital pharmacy, intensive care unit and neurology department. In addition, the pharmaceutical company acting as sponsor, marketing authorization holder and/or manufacturer takes an important part in the hospital circuit, which is a first for a drug. Thus, an increased coordination is essential to ensure safety and quality of these medicines.

Another particularity of CAR-T cells is their restricted use to qualified centers. Pharmaceutical companies, who decide whether the center is able to use their drug, currently perform this qualification. This is the first time for drugs, either. Even if circuits are similar, each company realizes its own qualification, according to its internal procedures. Thus, the establishment of a regulatory framework was necessary. In 2019, the French Ministry of Health published a decree limiting the use of autologous CAR-T cells to certain hospitals. This decree offers to involved actors a regulatory framework for the use of autologous CAR-T cells.

Briefly, the circuit is organized as following. Peripheral blood mononuclear cells (PBMC) containing lymphocytes T are collected by apheresis from the patient. At this step, collected cells correspond to the starting material that will be used for the manufacturing of the CAR-T cells drug. According to the French regulation, cell handling is under the responsibility of a CTU, authorized by the French Medicines Agency. Thus, collected cells are transferred from the apheresis unit to the CTU, where two circuits are possible: (i) cells shipment to the manufacturer at 4°C as a cell suspension, or (ii) cells cryopreservation in a nitrogen vapor phase before the shipment to the manufacturer. Then, manufactured CAR-T cells become an Advanced Therapy Medicinal Product (ATMP) in application of EU regulation 2001/83/EC. In France, all medicines including ATMP are under the responsibility of the hospital pharmacy. Thus, the final product must be send to the hospital pharmacy, where the pharmacist is responsible of reception steps. Currently, all manufactured CAR-T cells are distributed to hospitals as cryopreserved products, with two possibilities for the receipt: (i) the pharmacy disposes a monitored cryobiology area and can store the product. (ii) the pharmacy establishes a partnership with the CTU, who will store CAR-T cells, under the responsibility of the hospital pharmacy.

Finally, when the patient is eligible to receive CAR-T cells, thawing will be performed by the pharmacy staff or, by the CTU staff under the responsibility of the hospital pharmacy. The transport to the clinical department is done by the pharmacy staff, within 30min for Kymriah and 3h for Yescarta. The thawed bag is delivered to the nurse, who will proceed to the infusion under medical prescription.

All circuit steps should be done under double eyes validation and all the entries are made for long term traceability. The neurology department and the intensive care unit can be solicited in case of severe side effects. All theses steps need coordination and information sharing and the easiest way remain the electronic mails even if pharmaceutical companies have implemented electronic platforms.

VIDEO DROP

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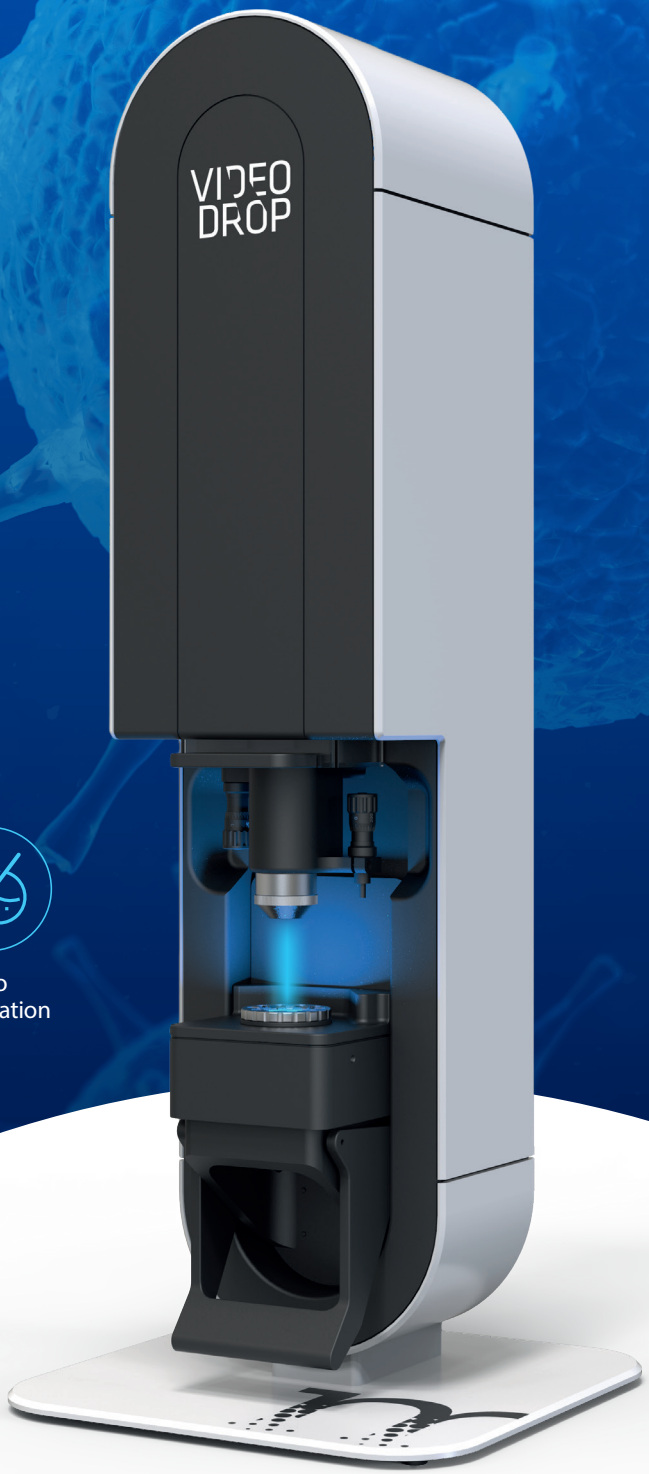

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Allogeneic CART cell therapy: first steps of a long journey

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Recent progress in understanding the cancer cell/immune system interactions during tumorigenesis has brought insights into the development of new era of cancer treatments. Beside unprecedent long term clinical responses in multiple cancer types, in particular solid tumors, obtained by checkpoint inhibitors (anti-PD1, anti-PDL1 and anti-CTLA4), other immunotherapies based on monoclonal antibodies and cancer vaccines have shown encouraging clinical responses. In parallel to this paradigm change triggered by the efficient stimulation of the patient's immune system, the development of Chimeric Antigen Receptor T-cell (CART), which give to the patients engineered T lymphocytes, has emerged as a potent and curative cancer therapy, notably in haematological malignancies.

The generation of patient-specific (autologous) CART cells (Fig.1) is allowed by the collection of the patient's own T-cells (by leukapheresis) and the introduction of synthetic proteins (CARs) through viral vectors (or with transposons/sleeping beauty systems). CARs are artificial receptors consisting of a fusion of a single chain variable fragment (scFv) with the intracellular signaling domains of the T-cell receptor (TCR) and one or more T cell co-stimulatory molecules, such as CD28, 4-1BB or OX-40. After being expanded ex-vivo, these "living drugs" are reinfused back into the patient to effectively recognize a tumor-specific antigen and kill tumor cells.

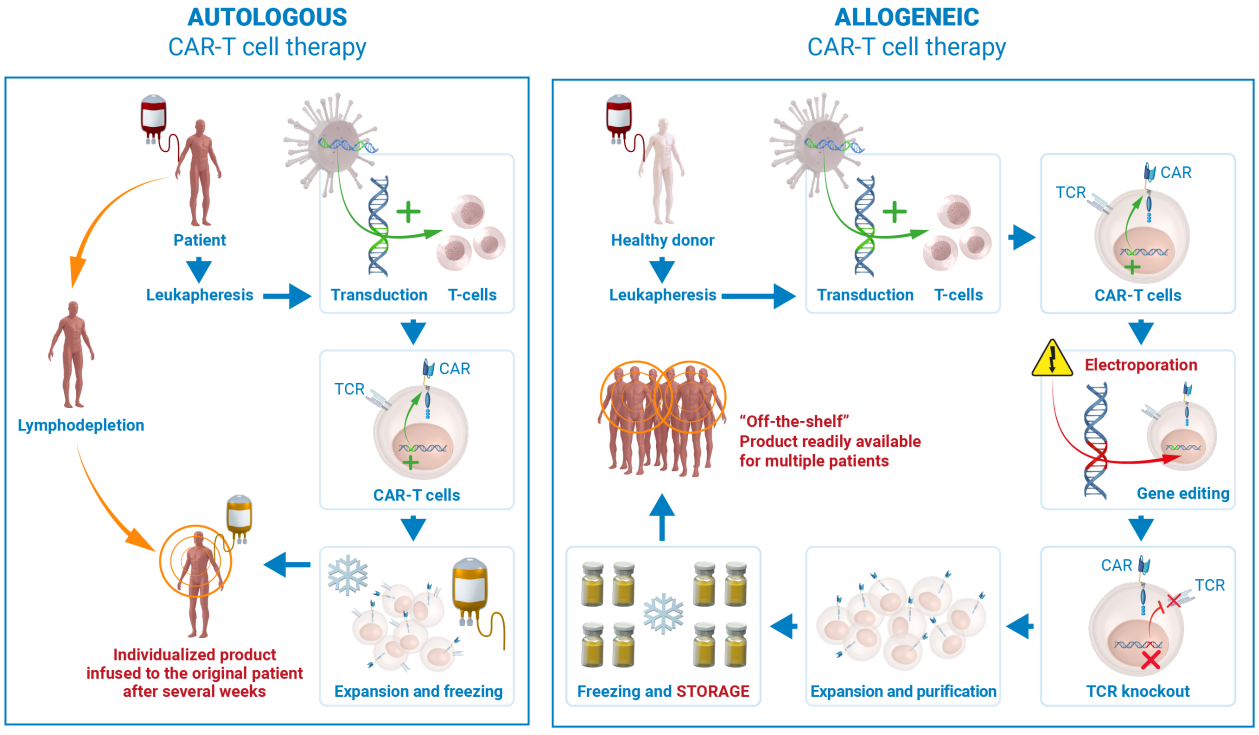

Figure 1: Autologous vs. Allogeneic CART cells

In the last 3 years three autologous CD19-directed CART cell products were approved: Kymriah® (tisagenlecleucel) (2017) and Yescarta® (axicabtagene ciloleucel) (2017), to treat relapsed/refractory (r/r) acute lymphoblastic leukemia (B-ALL) and Non-Hodgkin's lymphoma (NHL) as well as Tecartus™ (brexucabtagene autoleucel) (2020) for r/r Mantle Cell Lymphoma (MCL). Despite remarkable clinical results, access to these new therapies remains limited due to multiple factors such as patient-specific nature (number and quality of patient T cells collected from apheresis), time required for manufacturing (3-4 weeks), limited product for redosing, complex logistics, risk of contamination from inadvertently transduced leukemic blasts and pricing.
Therefore, alternative approach using "off-the-shelf" allogeneic CART cells remains highly attractive to provide better scale, standardized process and readily available product to treat patients, simpler logistics and lower costs.

Off-the-shelf CART cell therapy

Making "off-the-shelf" CART cell therapy involves the use of T cells from healthy donors. This provides a starting material with an optimal fitness allowing multiple gene editing and engineering possibilities to extend the reach of CART cells to more patients and cancers beyond hematological malignancies. However, to achieve all these promises, allogeneic CART cells are facing two major issues: graft-versus-host disease (GVHD), which can lead to patient's healthy cells damage and the opposite problem, host-versus-graft rejection of the CART cells due to HLA mismatches between donors and recipients.
To overcome the first hurdle, CART cell developers took advantage from advances in vector systems and genome editing to eliminate or dampen the expression of the TCRαβ chains (responsible for the GVHD reaction) at the surface of CART cells. Products using a variety of DNA nucleases (Table 1), small interfering RNA or inhibitory proteins targeting one or both TCR chains are under active preclinical and clinical development. Other strategies aiming to generate allogeneic CAR products with reduced risk of GVHD, such as the use of HLA-matched donor derived allogeneic hematopoietic stem cell transplant (HSCT), "off-the-shelf" virus specific memory T cells and alternative effector cells (γδT, iNKT, macrophages) with less allogeneic potential have also been implemented (Table 2) (1).

	Nuclease	Recognition site	Binding region	Targeting constraints	Off-target effects	Ease of targeting multiple gene	New target adaptability
Zinc- finger nucleases (ZFNs)	FokI	18-36 bp per ZFN pair	Zinc-finger protein	Difficult to target non-G-rich sites	Moderate	Low	Low
Transcription activator-like effector nucleases (TALENs)	FokI	28-40 bp per TALEN pair	RVD tandem repeat region of TALE protein	5' targeted base must be a T for each TALEN monomer	Low	Low	Moderate
The clustered regularly interspaced short palindromic repeats (CRISPR)	Cas9	22bp and up to 44 bp for double nickase	Single-strand guide RNA (gRNA)	Targeted site must precede a PAM sequence	Variable	High	High
MegaTAL Nucleases Protein Engineered I-CreI Homing endonuclease	Mega-nuclease	14-40 bp	Target recognition domain	Only pre-existing meganuclease recognition site	Low	Low	Low

Table 1: Summary table of different gene editing techniques used to generate "off-the-shelf" CART cells

Source of cells	Advantages	Limitations
Allogeneic Hematopoietic Stem Cell Transplant (HSCT) Donor	-Generated from the original Hemopoietic Stem Cell Transplantation (HSCT) donor - Donors are HLA matched to patients - Less likely to cause GVHD	- Limited to patients who have received an allogeneic HSCT - Need to be manufactured for each individual patient
"Off the Shelf" Virus Specific memory T Cells	- Known specificity of the TCR of theses memory T cells - Less likely to cause GVHD - Cells did persist up to 90 days in patients	- TCR and CAR co-activation may limit T-cell function - Partial HLA matching to patients - Manufacturing process longer than autologous CARs (5 to 6 weeks)
Alternative Effector (non- αβ T) Cells	- Produced from different cytotoxic cells with low GVHD properties such as: NK cells that do not require HLA matching 'Invariant'NKT (iNKT) cells that have a restricted TCR γδT cells that do not have an MHC restricted TCR activation Macrophages which are central effectors of the innate immune response	- Large scale manufacturing process is not optimal for all these cell types (paucity of cells, long expansion phase)
Gene-Edited Healthy Donor T cells	- Could be generated by different gene editing technologies ZFN, TALEN, megaTAL nucleases and CRISPR/Cas9 systems - Deletion of the T cell receptor constant α chain (TRAC) gene avoids GVHD - Knock-in of the CAR construct into the TRAC locus by homologous recombination allows TCR-like regulation of CAR expression - Current manufacturing processes produce >99% CAR+ cells	- Partial to no HLA matching to patients - Remaining TCR+ cells may cause GVHD at high doses (>5x104 /kg TCR+ cells)

Table 2: Strategies to deploy allogeneic CART Cells

Currently, there are eighteen trials evaluating the use of allogeneic CART cells (Table 3), with some phase I data showing promising anti-tumor activity in CD19+ B-cell malignancies with low to no GVHD. However, these trials are also highlighting several challenges that have still to be overcome before a large deployment of an efficient and safe "off-the-shelf" CART cell therapy. First, how long allogeneic CART cells need to persist to ensure a deep remission in different malignancies? Second, how to make these cells persist long enough to expand and achieve greater efficacy? Third, as in the setting of CLL, autologous CD8+CD27+CD45RO- CART cell phenotype was linked to more potent anti-leukemic activity (2), whether this T cell subpopulation or other T cell phenotype is required for efficient allogeneic CART therapy has still to be determined? Finally, how to ensure safety of these edited cells?

To prevent the early rejection of infused allogeneic CART cells by the recipient immune system, strategies aiming to disrupt HLA-class I and class II molecules expression on CART cells, via disruption of genes such as beta-2-microglobulin (β2M) and regulatory factor X complex (RFX5) have been validated in preclinical studies (3, 4). Alternative strategies combining β2M knock-out (KO) and overexpression of inhibitory signals such as non-polymorphic HLA class I molecules (HLA-E and HLA-G) have also been developed to address the issue of natural killer (NK) cell-dependent lysis (5). However, these strategies need to be clinically tested in order to determine the best option.

Table 3: Open clinical trials with allogeneic CAR T cells

Target	Disease	ClinicalTrial.gov identifier	Sponsor	Drug product
CD19	R/R CD19+ leukemia & lymphoma	NCT03166878	Chinese PLA General Hospital (China)	CD19-directed BBζ CAR-T cells (termed UCART019) by combining the lentiviral delivery of CAR and CRISPR RNA electroporation to disrupt endogenous TCR and B2M genes
	R/R B- ALL adult	NCT02746952	Institut de Recherches Internationales Servier (France)	UCART19* (Allogeneic Engineered T-cells Expressing Anti-CD19 Chimeric Antigen Receptor
	R/R B- ALL pediatric	NCT02808442	Institut de Recherches Internationales Servier (France)	UCART19* (Allogeneic Engineered T-cells Expressing Anti-CD19 Chimeric Antigen Receptor
	R/R large B cell or follicular lymphoma	NCT03939026	Allogene Therapeutics (USA)	ALLO-501* is an allogeneic CAR T cell therapy targeting CD19
	R/R NHL or B- ALL	NCT03666000	Precision BioSciences (USA)	PBCAR0191, allogeneic anti-CD19 CAR T Cells
	R/R large B cell lymphoma	NCT04416984	Allogene Therapeutics (USA)	ALLO-501A* is an allogeneic CAR T cell therapy targeting CD19 ALLO-647 is a monoclonal antibody that recognizes a CD52 antigen
	R/R B cell malignancies	NCT04035434	CRISPR Therapeutics (USA)	CTX110 (CD19-directed T-cell immunotherapy comprised of allogeneic T cells genetically modified ex vivo using CRISPR-Cas9 gene editing components
	R/R B cell malignancies	NCT03229876	Shanghai Bioray Laboratory (China)	CD19-UCART (Allogeneic Engineered T-cells Expressing Anti-CD19 CAR)
	R/R B-cell Malignancies	NCT01430390	Memorial Sloan Kettering Cancer Center (USA)	In Vitro Expanded Allogeneic Epstein-Barr Virus Specific Cytotoxic T-Lymphocytes (EBV-CTLs) Genetically Targeted to the CD19 Antigen
	R/R adult and pediatric B-cell ALL after HSCT	NCT03389035	Fondazione Matilde Tettamanti Menotti De Marchi Onlus (Italy)	CARCIK-CD19 Allogeneic (donor-derived) Cytokine Induced Killer (CIK) cells transduced with a transposon CD19 Chimeric Antigen Receptor (CAR) gene
	R/R B- ALL	NCT04173988	Children's Hospital of Fudan University (China)	CD19-Directed Allogeneic Chimeric Antigen Receptor T- cell (alloCART-19)
CD22	R/R B- ALL	NCT04150497	Cellectis S.A (France)	UCART22 Allogeneic engineered T-cells expressing anti-CD22 CAR
CD19 and CD20 or CD22	R/R B leukemia or lymphoma	NCT03398967	Chinese PLA General Hospital	Universal Dual Specificity CD19 and CD20 or CD22 CAR-T Cells
CD123	R/R AML	NCT03190278	Cellectis S.A. (France)	UCART123 Allogeneic engineered T-cells expressing anti-CD123 Chimeric Antigen Receptor
BCMA	R/R Multiple myeloma	NCT03752541	Shanghai Bioray Laboratory (China)	BCMA-UCART
	R/R Multiple myeloma	NCT04093596	Allogene Therapeutics (USA)	ALLO-715* is an allogeneic CAR T cell therapy targeting BCMA
CS1	R/R Multiple myeloma	NCT04142619**	Cellectis S.A. (France)	UCARTCS1A
NKG2D ligands	Unresectable metastatic colorectal cancer	NCT03692429	Celyad (Belgium)	CYAD-101 targeting of NKG2D-ligands

B- ALL, B cell acute lymphoblastic leukemia; BCMA, B cell maturation antigen; CAR, chimeric antigen receptor; NHL, non- Hodgkin; AML, Acute myeloid leukemia.
* UCART19, ALLO-501A & ALLO-715 use Cellectis' technologies

** on hold since 6/07/2020.

In parallel, given the success of lymphodepleting chemotherapies in increasing the engraftment and expansion of autologous CART cells, intensification of patient lymphodepletion may be enough to mitigate acute rejection of non-HLA matched CART cells. In this case, gene editing, and cell engineering methods have been used to make infused allogeneic T cells resistant to lymphodepleting agents such as alemtuzumab (anti-CD52 monoclonal antibody) or purine analogues. As a proof-of-concept, phase I clinical data of two trials evaluating UCART19* (an "off-the-shelf" product with CD52 KO) in adult and pediatric B-ALL, highlight the importance of an intense lymphodepletion for the efficacy of allogeneic CART therapies (6).
However, it is important to underline the need for additional studies to optimize the combination, depth and duration of lymphodepleting regimens in order to balance the requirement for patient immunosuppression against the risks of lymphopenia and the associated viral reactivation and infectious complications.

Given the increasing number of clinical trials testing autologous and allogeneic CART cells in different tumor settings and subsequent translational data, the introduction of innovative tools such as single cell RNA sequencing to study cell intrinsic properties and the sustainable development of gene editing platforms, "off-the-shelf" CART therapy should rapidly overcome its major limitations to offer new perspectives of treatment to a large population of patients with a cost similar to biologics.

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CAR –T cells for solid tumors

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Worldwide, cancer is the second most frequent cause of death after cardiovascular diseases. Over the past decade, immunotherapy (immune checkpoint inhibitors (ICI)) led to a significant breakthrough in patient management, allowing durable response and survival for several tumor types. However, around 30% of patients achieve tumor response (all tumor types) with anti-PD-(L)1 monotherapy, and in the responder patients, about 60% will develop secondary resistance (1). Although ipilimumab plus nivolumab gave impressive results (from 36 to 58% of objective response) in lung, renal, and melanoma cancer, there is an urgent need to improve immunotherapy efficiency using new combinations or new treatment strategies.
CAR T-cells are genetically modified T-cells expressing the chimeric-antigen receptor (CAR) that enables them to specifically recognize and bind the target tumor antigen in an HLA-independent manner. This specific recognition is followed by cytotoxic destruction of the target cells through perforin/granzyme-induced apoptosis (2).
CAR T-cells towards CD19 have already revolutionized the treatment of relapsed and refractory acute lymphoblastic leukemia (ALL) and B-cell non-Hodgkin's lymphoma (NHL) (3,4). However, in the case of solid tumors, CAR-T cells' use encounters several limitations.
Indeed, even considering a unique tumor type, response to specific immunotherapies may be very heterogeneous. Tumors could be divided into three categories that could predict immunotherapy response(5): 1/»inflamed,» with enriched T-cells located mostly in tumor parenchyma; 2/»immune-excluded», with enriched T-cells located within dense tumor stroma that prevents T-cell trafficking into parenchyma even upon ICI therapy; and 3/»immune desert» tumors lacking T-cells.
Data on solid tumors treatment with CAR T-cells are already available but limited to several case reports or small phase I/II clinical trials with poor results (6–14). Some drugs may slow tumor progression, but no durable response and survival were induced. The CAR T cells underwent rapid loss of functional activity that limited their therapeutic efficacy. Other phase I/II clinical trials are still ongoing (Table 1a). Researches try to circumvent the principal barriers to successful clinical application of CAR cell therapy in solid tumors:

(1) Impaired molecular trafficking into the tumor: Forced upregulation of chemokine receptors is particularly pertinent to attract lymphocytes to the tumor site (15–17). Desmoplasia could also block T cells' entrance in tumor site (2,18). Heparanase is an enzyme that degrades heparan sulfate proteoglycans (HSPGs), a significant component of desmoplasia. Engineering CAR-T cells to express heparinase enhanced tumor T-cell infiltration and antitumor activity in vitro and in vivo mice models (16).

(2) Microenvironment-mediated alterations include the presence of immunosuppressive cells that secret several immunosuppressive cytokines/chemokines and soluble factors and/or express checkpoint inhibitor ligands, leading to T cells exhaustion. Thus, engineered CAR T cells must be armed to circumvent the tumor immunosuppressive microenvironment. Methods to achieve this goal include an additional stable knock-out of inhibitory receptors and constitutive expression of a

costimulatory ligand (19)(15). A novel extension of these approaches uses engineered CART cells to produce and deliver anticancer drugs such as cytokines of the IL12 family upon stimulation with the target antigen, enhancing cytotoxic T cell function and simultaneously inhibits immunosuppressive processes (16,20). Anti-CTLA-4 antibody leads to selective depletion of Tregs within tumor lesions in mice, underlining another argument for its combined use with CAR-T cell infusion to enhance engineered T-cell treatment(16). The combination of PD-1 or CTLA-4 checkpoint blockade antibodies with CAR-T cell therapy in different tumors is currently investigated within clinical trials (NCT04003649) (16).

One other approach to counteract the multitude of redundant immunosuppressive signals in the tumor microenvironment is to convert Th2 to Th1 microenvironment and attract other antitumor immune cells. Cytokines are logical choices to achieve this goal (20). The basic idea is put to practice by using so-called 'TRUCKs', CAR redirected T cells (Fourth generation CAR T cells) that deliver a transgenic product ('payload') to the targeted tumor tissue(21). CAR T cells are engineered with a nuclear factor of the activated T cell (NFAT)-responsive expression cassette for the inducible expression of a transgenic cytokine(21) (Figure 1). Chemokines or cytokines such as IL-15(20), IL-12(22), IL-18, or both (21) are delivered to the tumor tissue to make sure that the product is deposited in the CAR targeted tissue with minimal systemic side effects (21,22). This approach shapes a favorable milieu for these cells. Some of these approaches could obviate the need for lymphodepletion pre-conditioning treatment in mice models, which is particularly interesting in the context of solid tumor immunotherapy.

(3) The choice of an efficient CAR cell construct permitting rapid and low-cost treatment, few side effects but long-lasting antitumor immunity: the production of autologous cells has many limitations in the clinical setting of solid tumor. «Off-the-shelf» allogeneic T cells could be an option but may cause severe graft-versus-host disease (GVHD)(23). One other option would be to use Natural killer (NK) cells, which are part of the innate immune system. They are naturally capable of killing tumor cells based on the balance between activating and inhibitory receptors' engagement. They also secrete an array of inflammatory cytokines that help establish an adaptive immune T-cell response and recruit other immune cells(24). This property may potentiate the activity of the CAR (25). Based on their cytotoxic properties, NK-92 has become a critical cell line for preclinical research. It is the only NK cell line approved by the US Food and Drug Administration (FDA) for phase I and II clinical studies and gave some preliminary results for treating advanced cancers (25). Therefore they constitute a suitable source of NK cells for CAR engineering since they are not responsible for GVHD because they do not require HLA matching and usually do not induce cytokine storms. NK-92 cell line has to be irradiated before infusion for safety (transformed cell line)(26). In the next future, more sophisticated safety measures like the expression of inducible caspase-9 as a suicide gene may thereby replace cells' irradiation (27,28).

Moreover, the CAR costimulatory domain used influences T cells phenotype. Indeed, co-stimulation with a CD28 domain is associated with a potent effector phenotype but a short duration of action. High levels of cytolytic activity and cytokine secretion are often responsible for cytokine release syndrome. On the other hand, CAR-T cells carrying a 4-1BB costimulatory domain tend to develop and persist longer in vivo, appear less prone to exhaustion. They could generate central memory T cells, which are essential for long term tumor growth control. Combining two costimulatory domains in a so-called 'third-generation' CAR provides superior antitumor activity and increases persistence in an in vivo CEA+ colon carcinoma murine model(21).

(4) The major barrier of CAR T cells is the difficulty for identification of a proper membrane expressed tumor-associated antigens: after initial tumor reduction by CAR T cells, antigen-negative cancer cells not recognized by CAR may give rise to tumor relapse due to selection of resistant tumor cells clones (21). In counterpart, off-target effects could limit CAR-T cells' use due to the lack of tumor specificity of the chosen target antigen. Various natural cytotoxicity receptors (NCRs) allow the recognition of tumors by NK cells. Chimeric receptors based on the extracellular domain of NCRs could be an exciting option to specifically target tumor cells with respect to normal cells (29,30).

Moreover, most tumors' proteins are expressed inside cells instead of at the cell surface (28% only), making them unavailable to act as antigens for CARs(31). One strategy to overcome this barrier is to engineer TCR-T cells. TCR-T cell interacts with a complex composed of MHC molecules that present an endogen peptide, leading to quicker destruction of tumor cells. Sofar, TCR-engineered T cells have shown more significant promises against solid tumors such as metastatic melanoma(32) than CAR-T cells. A recent clinical trial (Abs #9523 ASCO 2019) using a TCR-T cell associated with an anti-CD3 single-chain variable fragment (tebentafusp) (33) gave some impressive results. These bispecific TCR-antiCD3 T cells recognize and bind gp100 endogen protein presented by MHC, allowing their activation for melanoma cells killing regardless of their intrinsic specificity. Responses were obtained in 24% of treated patients with 10 % of complete response and only 2.4% of drug-related AEs leading to drug discontinuation. These promising results must be confirmed in upper phase clinical trials. Finally, to overcome «on-target, off-tumor» toxicity, one approach is to add an inhibitory CAR molecule targeting an antigen on normal tissue but not on the tumor to the TCR-T cell. Knocking down the endogenous TCR could be another option. The remaining barriers, such as the immunosuppressive microenvironment, the lack of lymphocyte chemoattraction for TCR T cells efficiency, are quite similar to CAR T cells and could be overcome with similar strategies(20).

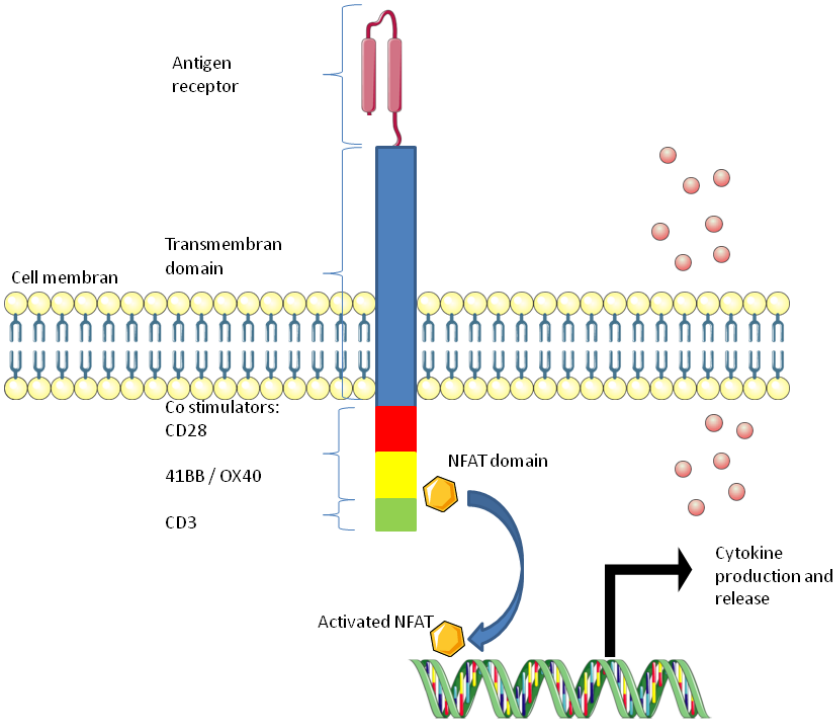

Fig. 1: Representation of TRUCK T cell: this cell is engineered with a chimeric receptor, which combines to costimulatory receptor: CD28 + 41BB or OX40 and an additional nuclear factor of the activated T cell (NFAT)-responsive expression cassette for the inducible expression of a transgenic cytokine after receptor engagement.

Clinical trial number	Targeted cancer type	CAR-targeted antigen	CAR construct	Expected advantage of this engineered cell
NCT03874897 Phase 1	Solid tumors; ei advanced gastric cancer, esophagogastric junction cancer, and pancreatic cancer	Claudin 18.2	2nd generation CD28 T cells	Claudin 18.2 postive cancers killing by own patients' engineered T cells following lymphodepletion regimen
NCT03941457 Phase 1/2	Pancreatic cancer that express ROBO1	ROBO1	3d generation CD28-41BB NK92cells	ROBO1 positive pancreatic cancer cells killing by CAR- engineered NK92 cells line for an Off-the-shelf treatment without lymphodepleting regimen
NCT03747965 Phase 1	Mesothelin positive solid tumor ; e.i pancreatic carcinoma, cholangiocarcinoma and ovarian cancer	mesothelin	CAR construct N/A CRISPR-Cas9 mediated PD-1 gene-knocked out CAR Tcell	Increase CAR T cells efficiency against mesothelin positive cancer by knocking out the inhibitory signal mediated by PD-1/ PD-L1 interaction, CAR T cells are infused after conditioning regimen
NCT03545815 Phase 1	Mesothelin positive solid tumors	mesothelin	CAR construct N/A CRISPR-Cas9 mediated PD-1 and native TCR gene-knocked out CAR Tcell	Increase CAR T cells efficiency against mesothelin positive cancer by knocking out the inhibitory signal mediated by PD-1/ PD-L1 interaction. Native TCR knock out increase mesothelin directed CAR T sensitivity and specificity.
NCT03615313 Phase 1/I2 NCT04503980 Phase 1 NCT04489862 Phase 1	Mesothelin positive solid tumors	mesothelin	CAR construct N/A Anti PD-1 producer CAR T cell	Increase CAR T cells efficiency against mesothelin positive cancer and secreting anti PD-1 antibodies at the tumor side which disrupt the inhibitory PD-1/ anti PD-1 axis. CAR T cells are infused after lymphodepleting regimen
NCT03932565	Nectin 4 positive solid tumors: non-small cell lung cancer, breast cancer, ovarian cancer, bladder cancer, and pancreatic cancer AND tumor associated fibroblast that express FAP gene	Nectin4/FAP	Fourth generation CAR T cells leading to cytokine secretion such as IL7 and CCL19 or/and IL12	This CAR could specifically kill nectin4 positive tumor as well as desmoplasia. IL7 and CCL19 secretion could increase CAR T cells infiltration and survival
NCT03373097 Phase 1/2	GD2 positive solid tumors such as neuroblastoma	GD2	CAR construct N/A Contains an inducible Caspase 9 gene	This CAR could specifically kill GD2 positive solid tumors. Inducible Caspase 9 gene could kill CAR T cells in case of relevant side effects. CAR T cells are infused after lymphodepleting regimen
NCT02992210 Phase 1/2	GD2 expressive solid tumors	GD2	GD2-scFv/CD28 4-1BB/ / CD3ζ- CD27 fused with iCasp9 (4SCAR-GD2)	This CAR T cell could specifically kill GD2 positive solid tumors. Inducible Caspase 9 gene could kill CAR T cells in case of relevant side effects.
NCT03618381 Phase 1	EGFR expressing non CNS solid tumors	EGFR	Arm A: second generation 4-1BBζ EGFR806-EGFRt ARM B: second generation 4-1BBζ EGFR806-EGFRt and a second generation 4 1BBζ CD19-Her2tG	ARM A: anti-EGFR CAR T could be available to kill EGFR positive tumor cells. In case of relevant adverse event, EGFRt (targeted by cetuximab) serves as a suicide mechanism for the elimination of the transferred T cell products. ARM B: This CAR Target EGFR expressing tumor cells and also CD19 to recognize B cells as APC that will promote the expansion and persistence of the CAR T cells. EGFRt and HER2tG targeted respectively by cetuximab and trastuzumab serve as suicide mechanisms in case of relevant toxicity

Tableau 1: Main ongoing clinical trials with CAR-T/NK cells for solid tumors (1/3)

Clinical trial number	Targeted cancer type	CAR-targeted antigen	CAR construct	Expected advantage of this engineered cell
NCT04483778 Phase 1	Non CNS solid tumors	B7-H3 (CD276; immune check point)	Arm A: second generation 4-1BBζ B7H3-EGFRt CAR T Arm B: second generation 4-1BBζ B7H3-CD19-Her2tG CAR T	CAR engineered T cells could kill B7H3 expressing tumor cells CAR targeting CD19 permit to increase antigen presentation by B cells that promote the expansion and persistence of the CAR T cells EGFRt and HER2tG targeted respectively by cetuximab and trastuzumab serve as suicide mechanisms in case of relevant toxicity
NCT04432649 Phase 1/2	B7-H3 (CD276) positive solid tumors	B7-H3 (CD276; immune check point)	B7-H3 scFv/CD28 4-1BB/ / CD3ζ- CD27 fused with iCasp9 (4SCAR-276)	This CAR could specifically kill B7-H3 positive solid tumors. Inducible Caspase 9 gene could kill CAR T cells in case of relevant side effects.
NCT04511871 Phase 1	HER2 positive solid tumors	HER2	CAR T construct N/A	CAR T cells that specifically recognize HER2 could kill HER2 positive tumor following lymphodepleting regimen
NCT03851146 Phase 1	Lewis Y positive solid tumors	Lewis Y	2nd generation CAR T (CD28 CD3 ζ)	CAR T cells that specifically recognize Lewis Y antigen could kill Lewis Y positive tumor following lymphodepleting regimen
NCT02932956 Phase 1 NCT04405778 Phase 1	Glypican 3 positive solid tumors	Glypican 3	CAR T construct N/A	CAR T cells that specifically recognize Glypican 3 antigen could kill Glypican 3 positive tumor following lymphodepleting regimen
NCT02744287 Phase 1/2	PSCA expressing solid tumors	PSCA	PSCA scFv CD3ζ + MyD88/ CD40 inducible costimulatory domain	This PSCA CAR T cell with inducible MyD88/CD40 is designed to provide a powerful boost to T cell proliferation and persistence, production of immunomodulatory cytokines that enable the CAR-T to override key immune inhibitory mechanisms, including PD-1 and TGF-beta.
NCT04025216 Phase 1	advanced TnMUC1+ solid tumors (triple negative breast cancer, epithelial ovarian cancer, pancreatic cancer, and non-small cell lung cancer) (Arm 1)	TnMUC1	TnMUC1 scFV 4-1BB CD3 ζ	CAR T cells that specifically recognize TnMUC1antigen could kill TnMUC1positive tumor, after lymphodepleting regimen
NCT03706326 Phase I/II	MUC1 positive advanced oesophagial cancer	MUC1	3 arms: 1/ : Anti-MUC1 CAR-T cells 2/ PD-1 knockout Engineered T cells 3/ CAR-T combined with PD-1 Knockout T cells	CAR T cells that specifically recognize MUC1 could kill MUC1 positive tumor cells. CAR T cells efficiency could be boost in association with PD-1 knockout engineered T cells.
NCT02498912 Phase 1	high-grade MUC16 ecto positive ovarian, primary peritoneal, or fallopian tube carcinoma	MUC16	MUC16 scFv CD28 CD3 ζ fIL12 E GFRt	This engineered CAR T cells recognize and kill MUC16ecto expressing cancers and secrete IL-12 which induces secretion of interferon-gamma, promotes the activation of natural killer cells (NKs), and induces cytotoxic T-cell responses against tumor cells, which may result in immune-mediated tumor cell death and inhibition of tumor cell proliferation. Moreover, EGFRt expression lead to a mechanism for eliminating CAR T cells in case of relevant side effects. CAR T cells are infused after or not lymphodepleting regimen

Tableau 1: Main ongoing clinical trials with CAR-T/NK cells for solid tumors (2/3)

Clinical trial number	Targeted cancer type	CAR-targeted antigen	CAR construct	Expected advantage of this engineered cell
NCT02915445 Phase 1	EpCAM-positive nasopharyngeal carcinoma or breast cancer	EpCAM	3d generation CAR T cell: EpCAM scFV CD28 4-1BB double co stimulatory domain CD3ζ	CAR T cells that specifically recognize EpCAM antigen could kill EpCAM positive tumor.
NCT04348643 Phase 1/2	CEA positive advanced solid tumors	CEA	CAR T cell construct N/A	CAR T cells that specifically recognize CEA antigen could kill CEA positive tumor.
NCT03356782 Phase 1/2	CD133 or GD2 or Muc1 or CD117 positive sarcoma	Sarcoma specific antigen expression (CD133, GD2, Muc1, CD117)	4SCAR-IgT that target different sarcoma antigen and is construct as 4SCAR with CD28/ 4-1BB/ CD3ζ- CD27 fused with iCasp9	This strategy combines antibodies and T cells to kill sarcoma cells.
NCT04003649 Phase 1	glioblastoma	IL13Rα2	IL13Ralpha2scFv 4-1BB-CD3 ζ T cells	Patients could receive nivolumab + ipilimimab 14 days before the first infusion of CART cell + nivolumab, or not. Immune check point combination could potentiate CAR T cell efficiency against IL13Ralpha2 positive gliobastoma

Tableau 1: Main ongoing clinical trials with CAR-T/NK cells for solid tumors (3/3)

Clinical trial number	Targeted cancer type	TCR - targeted antigen	Expected advantage of this engineered cell
NCT03778814 Phase 1	Solid tumors especially lung cancer	Monoclonal TCR against tumor is selected by coculture with organoid tumor tissue from the interested patient and its TIL. After TCR selection, T cells from patients' PBMC are transected for anti tumor specific TCR expression	The expected results is an increased specificity and sensitivity of the own patient lymphocytes for his/her own tumor.
NCT03970382 Phase 1	incurable or metastatic solid tumors of the following types: melanoma, UC, ovarian cancer, colorectal cancer, breast cancer (HR+), or prostate cancer	NeoTCR P1 is composed of apheresis derived CD8 and CD4 T cells that are precision genome engineered to express one autologous TCR of native sequence that targets a neoepitope (neoE) presented by human leukocyte antigen (HLA) receptors exclusively on the surface of that patient's tumor cells and not on other cells in the body.	This clinical trial test NeoTCR P1 alone (arm A) or in combination with anti PD-1 nivolumab (ARMB) that could increase T cell efficiency by blocking the inhibitory axis PD-1/PD-L1
NCT02869217 Phase 1	Advanced solid tumors that express NY-ESO-1. Patients must be positive for HLA-A*02:01 or HLA-A*02:06	NY-ESO-1	Own patients' T cells are engineered to express NY-ESO-1 specific TCR to specifically kill their tumor cells which express NY-ESO-1 antigen following lymphodepleting regimen
NCT03247309 Phase 1	Advanced solid tumors that express MAGEA4/8	MAGEA4/8	Own patients' T cells are engineered to express MAGEA4/8 specific TCR to specifically kill their tumor cells which express MAGEA4/8 antigen following lymphodepleting regimen
NCT03686124 Phase 1	Advance d solid tumor, such as refractory melanoma. Patient's tumor must express tumor antigen by qPCR using a fresh tumor biopsy specimen	preferentially expressed antigen in melanoma (PRAME)	Own patients' T cells are engineered to express PRAME specific TCR to specifically kill their tumor cells which express PRAME, following lymphodepleting regimen With (grp 2) or without (grp 1) atezolizumab (anti PD-L1), which could improve T cells efficiency by blocking PD-1/PD-L1 inhibitory axis.
NCT03441100 Phase 1	Advance d solid tumor, such as refractory melanoma. Patient's tumor must express tumor antigen by qPCR using a fresh tumor biopsy specimen	MAGEA1	Own patients' T cells are engineered to express MAGEA1 specific TCR to specifically kill their tumor cells which express MAGEA1 antigen following lymphodepleting regimen
NCT03139370 Phase 1	(HLA)-DPB1*04:01 positive patients whose tumors are MAGE-A3 and/or MAGE-A6 positive	MAGEA3 and/or 6	Own patients' T cells are engineered to express MAGEA3/6 specific TCR to specifically kill their tumor cells which express MAGEA3/6 antigen following lymphodepleting regimen
NCT03515551 Phase 1/2	Advanced cancers which express NY-ESO-1 and/or LAGE-1A Patients must be HLA-A*0201 positive	NY-ESO-1 and/or LAGE-1A	Own patients' T cells are engineered to express NY-ESO-1 and/or LAGE-1A specific TCR to specifically kill their tumor cells which express NY-ESO-1 and/or LAGE-1A antigen
NCT04262466 Phase 1/2	HLA-A*02:01 positive patients PRAME positive tumor	PRAME immune-mobilizing T cell receptor against cancer (ImmTAC ®): PRAME specific TCR (targeting end) associated with an anti CD3 scFv(effector end)	ImmTAC ®structure recognizes and binds PRAME endogen protein presented by MHC, permitting their activation for tumor cells killing regardless of their intrinsic specificity. Engineered T cells efficiency could be increase in association of anti PD-L1 (arm2 phase 1) by blocking the inhibitory PD-1/PD-L1 axis.

Tableau 2: Main ongoing clinical trials with TCR-T cells for solid tumors (1/2)

Clinical trial number	Targeted cancer type	TCR - targeted antigen	Expected advantage of this engineered cell
NCT03973333 Phase 1/2	HLA-A*0201-positive Patients With Advanced MAGE-A4-positive Cancer	MAGEA4 immune-mobilizing T cell receptor against cancer (ImmTAC ®): MAGEA4 specific TCR (targeting end) associated with an anti CD3 scFv (effector end)	ImmTAC ®structure recognizes and binds MAGEA4 endogen protein presented by MHC, permitting their activation for tumor cells killing regardless of their intrinsic specificity. Engineered T cells efficiency could be increase in association of the anti PD-L1 atezolizumab by blocking the inhibitory PD-1/PD-L1 axis.
NCT04318964 Phase 1	HLA-A * 02:01 positive With Advanced NY-ESO-1 positive Cancer including soft tissue sarcoma	NY-ESO-1	Own patients' T cells are engineered to express NY-ESO-1 specific TCR to specifically kill their tumor cells which express NY-ESO-1 antigen such as soft tissue sarcoma after lymphodepleting regimen
NCT02650986 Phase 1/2a	NY-ESO-1 positive advanced solid tumor such as melanoma, ovarian, primary peritoneal or fallopian tube carcinoma, advanced synovial sarcoma which are positive for NY-ESO-1 (except for cohort 4) Patients must be positive for HLA-A*0201 (HLA-A2.1)	NY-ESO-1	The immunosuppressant the transforming growth factor beta (TGF-β) is produced by tumor cells and plays a key role in the repression of the immune system. These TCR T cells are transduced with a retroviral vector encoding a gene for a dominant-negative form of theTGF β receptor, TGFβDNRII, with potential immunomodulating activity. Upon administration, the NY-ESO-1 TCR/dnTGFβRII transgenic autologous T cells recognize and kill tumor cells that express NY-ESO-1. The expression of TGFβDNRII allows for the T cells to be resistant to TGF- β -mediated inhibition of T cell proliferation and activation, which allows optimal anti tumor T cells activity (after lymphodepleting regimen)
NCT03967223 Phase 2	HLA-A*02+ participants with NY-ESO1+ advanced metastatic or unresectable synovial sarcoma	NY-ESO-1	Own patients' T cells are engineered to express NY-ESO-1 specific TCR to specifically kill their tumor cells (synovial sarcoma) which express NY-ESO-1 antigen after lymphodepleting regimen
NCT03449108 Phase 2	ovarian cancer, anaplastic thyroid cancer, osteosarcoma, or other bone and soft tissue sarcomas that do not respond to treatment (refractory) or that has come back (relapsed)	Own patients' tumor antigens	LN-145 is made by collecting and growing specialized T-cells that are collected from the patient's tumor (TILs). LN-145-S1 is made using a modified process that chooses a specific TCR. The T cells may specifically recognize, target, and kill the tumor cells after lymphodepleting regimen followed by IL2 infusion

Tableau 2: Main ongoing clinical trials with TCR-T cells for solid tumors (2/2)

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Finding the T Cells that Matter:

Directly Link CAR-T Cell Phenotype and Function to Genotype

Chimeric antigen receptor T cell (CAR-T) therapy offers an exciting method for fighting and eradicating cancerous cells but is based on adoptive transfer of cells that are intrinsically heterogeneous. Our understanding of CAR-T cell therapy is still in its infancy, and current technologies limit the type of information we can gather to better understand why some constructs are efficacious and others are not. To develop more advanced and impactful therapies, it will be critical to link key indicators of success, such as cytokine secretion profiles, proliferation, and killing kinetics, to genotype and gene expression at a single cell level. Current technologies, however, afford only a partial view of the whole picture and we often have to rely on one-size-fits-all proxies for T cell activation, such as IFNγ secretion.

Linking cytokine secretion profiles and tumor cell killing to proliferation to build tailored QC assays

Advanced technology like the Berkeley Lights Beacon® and Lightning™ systems allow us to better characterize CAR-T cells at a single-cell level during process development. By providing a better understanding of their complex biology, these systems may enable us to create tailored QC assays that measure therapy-specific mechanisms

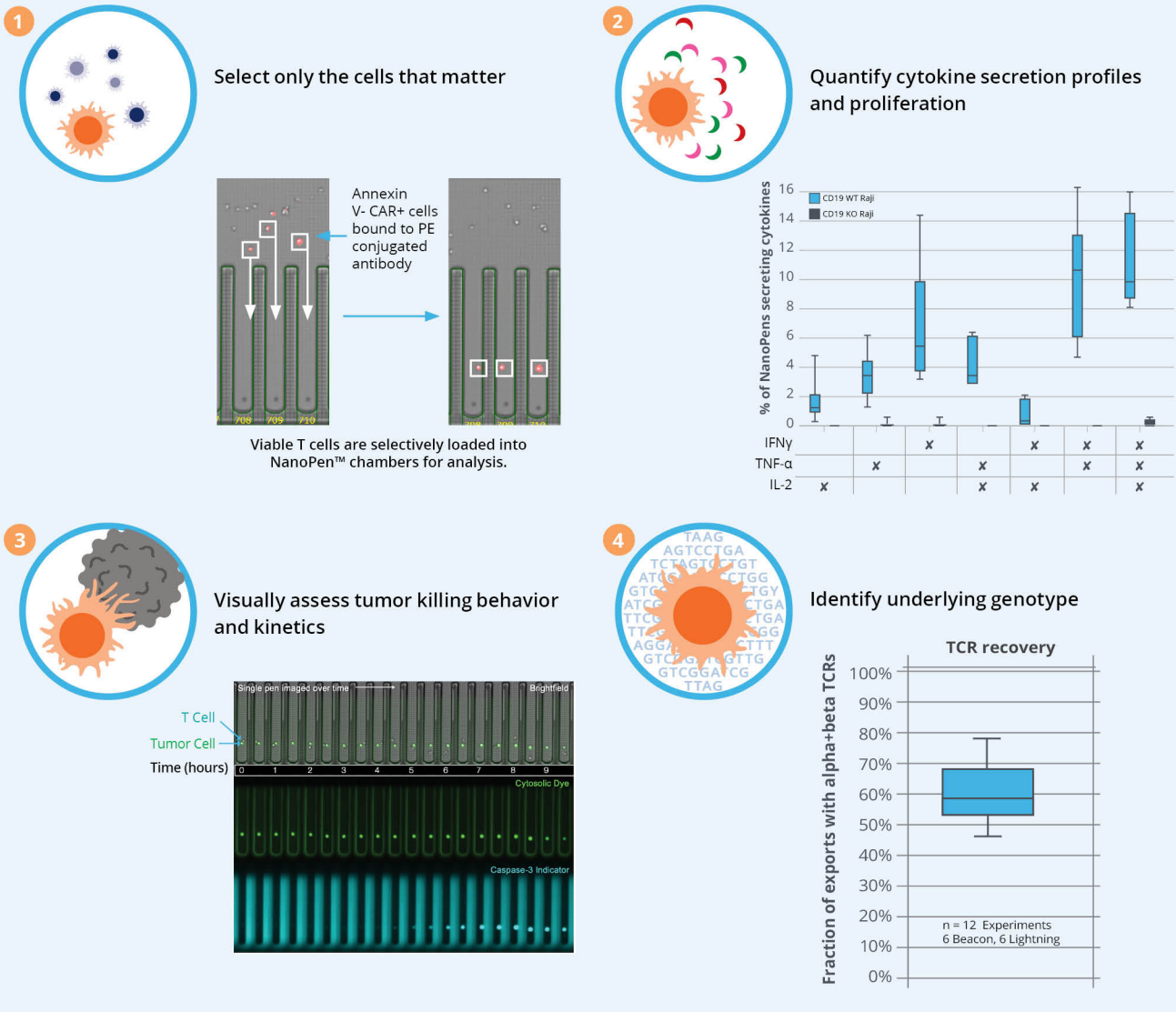

Figure 1. Directly linking phenotype and function to genotype at a single cell level.

of action and deliver more efficacious therapies. This technology has already been adopted to rapidly identify antibody therapeutics[1] and to generate cell lines with >99% monoclonality assurance[2].

The Beacon and Lightning systems use light and semiconductor technology to move single cells or assay beads into thousands of nanoliter-size chambers on a microfluidics chip. Single cells can be selectively placed into these chambers based on fluorescence profile or cell size and incubated with tumor cells (**Figure 1, Step 1**). After in-chamber co-culture, assay reagents can be perfused in and out of the chip to assess T cell proliferation and secretion of multiple cytokines of choice (**Figure 1, Step 2**). After cytokine secretion profiles have been mapped, you can link them to tumor cell killing kinetics at a single-cell level by monitoring tumor cell death over time (**Figure 1, Step 3**). Once profiled, cells of interest can be exported for further downstream analysis, such as sequencing (**Figure 1, Step 4**).

Making the most of precious samples to understand clinical success

A complicating factor in understanding why some CAR-T therapies succeed and others fail is the limited size of precious patient samples. Once CAR-T cells are administered to a patient, it is important to understand what contributes to a successful therapy. Blood samples collected 2 months after patients received CAR-T cell therapy can contain less than 10 CAR-T cells per microliter[3], but traditional analytical processes typically require hundreds of thousands of input cells. Even without the limitations of precious sample size, the information you can gather with current technology, such as FACS or sequencing, is isolated to a few characteristics per sample, but not the full picture. The Beacon system allows users to generate comparable data to FACS from up to 10x fewer cells (**Figure 2**), incubate individual T cells with tumor cells, and run multiple assays on the same cell to define cytokine profiles, assess proliferation, and link all of these key characteristics directly to tumor cell killing kinetics (**Figure 1, Step 3**).

This allows you to utilize small sample sizes more efficiently to better characterize and create better T cell therapies.

You can learn more by contacting Dr. Jean-Baptiste Pénigault or by visiting **berkeleylights.com/tcells**.

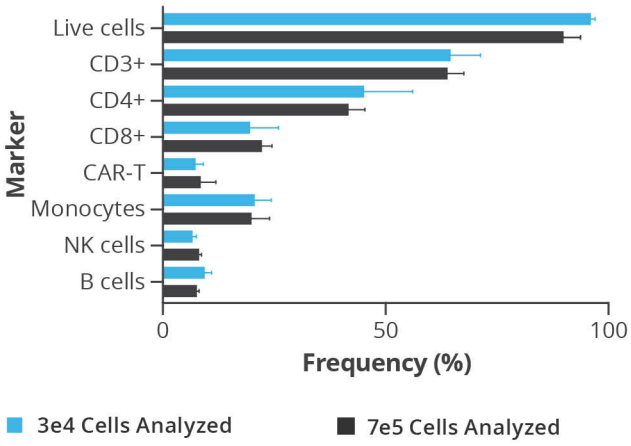

Figure 2. More data from fewer cells. A mock CAR-T cell therapeutic was generated and populations were analyzed using a Berkeley Lights system (■) or by standard flow cytometry (■).

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Learn More from One of Our Experts

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CAR-T CELLS IN DIFFUSE LARGE B-CELL LYMPHOMA: STATE OF ART

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INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) and other non-Burkitt aggressive B-cell non-Hodgkin lymphomas (NHL) are curable with front-line immunochemotherapy. However, patients with relapsed or refractory (R/R) disease face poor outcomes.1 Patients unable to achieve a response to second line treatment including platinum-containing salvage regimens or who relapse after high-dose therapy and autologous stem cell transplantation had limited options prior to the approval of CD19-specific chimeric antigen receptor T-cell therapy (CAR T-cell). 2,3

CAR T-CELL DEVELOPMENT IN DLBCL

CAR-T cells are autologous T-cells with an engineered T-cell receptor, which artificially targets surface tumoral antigen. Two autologous CD19 CAR-T cells are currently commercially available to treat patients with R/R DLBCL, both containing CD3ζ activation domain but with different co-stimulation domain (4-1BB or CD28) for expansion and persistence. This approach is on active use since April 2017 in US and since June 2018 in Europe for R/R B-cell malignancies, after pivotal Phase I-II studies of anti CD19 CAR-T cells were published2,3. Currently commercial available CAR-T are Tisagenlecleucel (tisa-cel, CTL019, 4-1BB costimulation)), indicated in R/R DLBCL and transformed follicular lymphoma (tFL) and acute lymphoblastic leukemia, and Axicabtagene-ciloleucel (axi-cel, KTE-X19, CD28 costimulation), authorized for R/R DLBCL, primary mediastinal B cell lymphoma (PMBL) and tFL.

Data from the pivotal trials to-date suggest 6-month ORR at 41-47%, and durable complete response (CR) rates around 30% to 40% in the three pivotal clinical trials (ZUMA1, JULIET, TRANSCEND), particularly for individuals with early CR. 2,3,4 In JULIET, the median overall survival (OS) was 12 months.3 In ZUMA1, with a median follow-up of 27.1 months, the median duration of response was 11.1 months (95% CI, 4.1 months to not estimable), 39% of patients remained in ongoing response, and the median OS was not reached.2 These 2-year follow-up data suggested that axi-cel and tisa-cel can induce durable remissions and meaningful OS benefit in patients with R/R aggressive B-cell lymphoma.

SAFETY PROFILE OF CAR TCELLS

Safety profile of CAR-T involves well-established toxicities, as CRS and ICANS. CRS is a systemic inflammatory response provoked by soluble mediators with wide clinical spectrum, ranging from mild flu-like syndrome to severe shock, observed grade III-IV in 13% to 23% after infusion. Besides CRS, ICANS is the other described complication, possibly involving an endothelial toxicity and causing variable neurologic signs, from slightly impaired cognitive function to coma or fatal seizures5. Grade > 3 ICANS have been reported in 12% of the patients treated with Tisa-cell and 28% in patients treated with Axi-cell. Both CRS and ICANS have been recently objected of a consensus for harmonizing gradings6. Other toxicities, despite often observed, are less reported such as hematologic toxicities.

In 2019 Fried et al. described 72% severe neutropenia and 28% severe thrombocytopenia in 29 responding patients affected by R/R B-cell malignancies treated by CAR-T7. In this study, cytopenias mostly occurred with a biphasic pattern. The first nadir of cytopenias seemed to be related to effects of lymphodepletion and activity of CRS, possibly mediated by macrophages; this could not explain the second occurrence of cytopenias observed at day 40 or more. As described for patients treated with Rituximab, a role for perturbation in stromal-derived factor 1 (SDF-1)/CXCligand 12 (CXCL12), a chemokine implicated in regulation of hemopoietic stem cells migration and activity, has been proposed7. As this chemokine has active role also in B-cell developing, possible interference has been hypothesized for normal granulocyte recovery8. We recently reported that G-CSF administration was safe and effective in reducing duration of hospitalization without impact on toxicities (CRS, ICANS) nor on anti-lymphoma activity of CAR-T with no impact of T-cell expansion.9

CAR T-CELL IN REAL LIFE

Clinical trials often have stringent eligibility criteria, and the outcomes observed in clinical trials may or may not be observed in real-life clinical practice because the study population in the clinical trials may not be representative of those treated in clinical practice. Several series have been published in US and in Europe. Seventeen US centers set out to delineate the characteristics and outcomes of 298 patients apheresed with intention to be treated with commercially available axicabtagene ciloleucel, an autologous anti-CD19 CAR T-cell.10 43% of patients had comorbidities or characteristics that would have deemed them ineligible to clinical trial. Despite this, safety and efficacy outcomes were comparable to ZUMA-1. Best overall and complete response rates in infused patients were 82% (95% CI, 77% to 86%) and 64% (95% CI, 58% to 69%), respectively. At a median follow-up of 12.9 months from the time of CAR T-cell infusion, median progression-free survival was 8.3 months (95% CI, 6.0 to15.1 months), and median overall survival was not reached. In UK, the results recently reported lower 3-month ORRs and CR rates of 37% and 21% for axi-cel and 29% and 17% for tisa-cel, respectively.11 In France, we recently reported a multicenter series of the 116 patients treated either with tisa-cel or axi-cel in 5 centers.12 The estimated 12-month progression-free survival (PFS) and overall survival (OS) were 47.2% (95%CI, 38.0 to 58.6) and 67.0% (95%CI, 57 to 79), respectively.

PERSPECTIVES

There have been a variety of innovations in the technical design of CAR-T cells, in an attempt to improve efficacy and reduce toxicity. The evolution of CAR design, with increasing complexity of structure will confer additional finesse to function. Another approach generating interest is multiple-antigen targeting, with a view to increase specificity, capture a variety of tumor clones, and reduce antigen-negative relapse. Fourth-generation “armored” CARs utilize a variety of techniques to combat an immunosuppressive microenvironment. This includes cytokine secretion by TRUCKs (T cell redirected for universal cytokine killing), of which the best studied example secretes IL-12 on encountering target antigen, so shifting the tumor microenvironment in favor of immune-activation and tumor cell killing. Combination with immunomodulating agents including checkpoint inhibitors are also under investigation.

CONCLUSION

CAR-T cells therapy represents a potential curative option for R/R DLBCL. The challenge in the future will be to select the right CAR-T products for the right patients at the right time. No doubt that the scientific and medical community will join their efforts to answer to this question.

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Litterature HIGHLIGHTS

Selection of scientific articles by our chiefs-editors

HIGHLIGHTS FROM LITTERATURE

Ibtissam Marchiq, Hélène Negre

I. Multiple myeloma:

After CD19 CAR-T success, recent results of the phase 1 clinical trial with b2121 B-Cell Maturation Antigen (BCMA) CAR T cells (funded by Bluebird bio and Celgene) in multiple myeloma (MM) showed at least 45% of complete response associated with CAR T cell expansion in vivo (1). Since this proof of concept, low BCMA expression on residual myeloma cells after BCMA CAR T treatment has been observed. Strategies combining multi-antigen-targeted CART can be employed to reduce antigen escape and increase efficacy (2). Two approaches are available:

- Polled CAR-T are a mixture of two CAR-T cells; each targeting different tumor antigens following the "OR" logic strategy. Their administration can be simultaneous or sequential.
- Dual CAR-T cells express two separate CAR T on each T cell.

Zah et al. recently published in Nature Communication, a preclinical study with a tandem single-chain bispecific CARs for multiple myeloma with BCMA and CS1 target based on OR-Gate strategy (3). This single-chain OR-gate CAR have superior CAR expression and proliferative capacity than the classic dual CAR-T targeting BCMA and CS1. In addition, administration of anti-PD-1 may increase the initial efficacy of the single-chain bispecific CAR T construction. Of note, further experiments would be needed to assess the long-term efficacy of the addition of anti PD-1. Two other targets expressed on plasma cells are also explored in pre-clinical studies;

- SLAM receptor CD229/LY9 CAR T cells show cytotoxic activity against MM-propagating cells without fratricide activity (4).
- GPRC5D protein is expressed on CD138+ MM cells from primary marrow samples; GPRC5D CAR T rapidly eradicates MM in mouse models with either a 4-1BB or a CD28 costimulatory signaling domain (5).

In addition to these targets for CAR T in MM, Stanley R. Riddell's team explored small-molecule GS inhibitors (GSIs) and showed that these adjuvants markedly increased surface BCMA levels in a dose-dependent fashion and improved tumor killing by CART cells in vitro (6). A clinical trial (NCT03502577) with the combination has started to enroll patients and the first results are expected in 2021.

1. Anti-BCMA CAR T-Cell Therapy bb2121 in Relapsed or Refractory Multiple Myeloma Noopur Raje, Jesus Berdeja, Yi Lin, Ph.D.,David Siegel, Sundar Jagannath, Deepu Madduri ,Michaela Liedtke, Jacalyn Rosenblatt, Marcela V. Maus, Ashley Turka, Lyh-Ping Lam, Richard A. Morgan, Kevin Friedman, Monica Massaro, Julie Wang, Ph.D.,Greg Russotti, Zhihong Yang, Timothy Campbell, Ph.D.,Kristen Hege, Fabio Petrocca, M. Travis Quigley, Nikhil Munshi, and James N. Kochenderfer ; NEJM (2019) 380;18.
2. Strategies combining multi-antigen-targeted CART cells Han et al. Journal of Hematology & Oncology (2019) 12:128 ;2- 10.
3. Systematically optimized BCMA/CS1 bispecific CAR-T cells robustly control heterogeneous multiple myeloma; Eugenia Zah, Eunwoo Nam , Vinya Bhuvan, Uyen Tran, Brenda Y. Ji, Stanley B. Gosliner, Xiuli Wang, Christine E. Brown & Yvonne Y. Chen ; Nature Communications (2020) 11:2283
4. CD229 CAR T cells eliminate multiple myeloma and tumor propagating cells without fratricide Sabarinath V. Radhakrishnan, Tim Luetkens , Sandra D. Scherer, Patricia Davis, Erica R.Vander Mause, Michael L. Olson , Sara Yousef, Jens Panse, Yasmina Abdiche, K. David Li, Rodney R. Miles, William Matsui, Alana L. Welm & Djordje Atanackovic ; Nature Communications (2020) 11:798

5. GPRC5D is a target for the immunotherapy of multiple myeloma with rationally designed CAR T cells Eric L. Smith, Kim Harrington, Mette Staehr, Reed Masakayan, Jon Jones, Thomas J. Long, Khong Y. Ng, Majid Ghoddusi, Terence J. Purdon, Xiuyan Wang, Trevor Do, Minh Thu Pham, Jessica M. Brown, Carlos Fernandez De Larrea, Eric Olson, Elizabeth Peguero, Pei Wang, Hong Liu, Yiyang Xu, Sarah C. Garrett-Thomson, Steven C. Almo, Hans-Guido Wendel, Isabelle Riviere, Cheng Liu, Blythe Sather, Renier J. Brentjens ; Science Translational Medicine (2019) 11:7746
6. -Secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. Margot J. Pont, Tyler Hill, Gabriel O.Cole, Joe J. Abbott, Jessica Kelliher, Alexander I.Salter, Michael Hudecek, MelissaL. Comstock, Anusha Rajan, Bharvin K. R. Patel, Jenna M. Voutsinas, Qian Wu, Lingfeng Liu, Andrew J. Cowan, Brent L. Wood, Damian J. Green, and Stanley R. Riddell; Blood (2019) ; 134:1585.

II. CARs beyond T cells:

To overcome logistic and clinical limitations linked to autologous CART cells, researchers from the University of Texas MD Anderson Cancer Center have engineered cord blood-derived allogeneic natural killer (NK) cells with a CAR that targets the CD19 antigen. These cells belong to the innate immune system and their recognition of tumor-associated antigens is independent of patients' HLA phenotype, which reduces the risk of GVHD after infusion. To further support the in vivo expansion and persistence of the transduced NK cells, a gene coding for interleukin-15 was introduced as well as a suicide switch gene (inducible caspase 9) to trigger apoptosis of the CAR-NK cells in case of toxicity in patients.
These triple-engineered NK cells were administered to 11 patients with relapsed or refractory CD19-positive cancers (Non-Hodgkin's lymphoma or chronic lymphocytic leukemia (CLL)). According to phase I/IIa trial results published in early 2020 (1), seven patients (64%) had a complete response and one showed partial remission. Responses were rapid and seen at all dose levels with no signs of toxic effects such as GVHD or cytokine release syndrome. Infused CAR-NK cells persisted at low levels for at least 12 months, however the use of post-remission therapy limits the assessment of the durability of response. Although promising, further clinical data will be needed to determine whether CAR-NK therapy could represent the next generation "off-the-shelf" adoptive cell therapy.

Researchers from the University of Pennsylvania have also proposed an alternative solution to overcome CART cell challenges. In a recent study, Klichinsky et al. (2) showed that genetically engineered human macrophages with CARs (CAR-Ms) demonstrated antigen-specific phagocytosis and tumor clearance in two solid tumor xenograft mouse models. Macrophages are central effectors of the innate immune system and play an important role in promoting an adaptive anti-tumor response. Engineering these cells to attack cancer represented a real challenge since macrophages are resistant to infection by the viral vectors commonly used in gene and cell therapy. The authors were able to solve this hurdle and transform CAR-Ms to highly inflammatory cells that could shape the surrounding tumor microenvironment and generate a vaccinal effect. Further studies will be important to consider how CAR-Ms could be implemented in cancer immunotherapy area.

1. Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. Enli Liu, David Marin, Pinaki Banerjee, Homer A Macapinlac, Philip Thompson, Rafet Basar, Lucila Nassif Kerbauy, Bethany Overman, Peter Thall, Mecit Kaplan, Vandana Nandivada, Indresh Kaur, Ana Nunez Cortes, Kai Cao, May Daher, Chitra Hosing, Evan N Cohen, Partow Kebriaei, Rohtesh Mehta, Sattva Neelapu, Yago Nieto, Michael Wang, William Wierda, Michael Keating, Richard Champlin, Elizabeth J Shpall, Katayoun Rezvani; N Engl J Med (2020); 382(6):545-553
2. Human chimeric antigen receptor macrophages for cancer immunotherapy. Michael Klichinsky, Marco Ruella, Olga Shestova, Xueqing Maggie Lu, Andrew Best, Martha Zeeman, Maggie Schmierer, Konrad Gabrusiewicz, Nicholas R. Anderson, Nicholas E. Petty, Katherine D. Cummins, Feng Shen, Xinhe Shan, Kimberly Veliz, Kristin Blouch, Yumi Yashiro-Ohtani, Saad S. Kenderian, Miriam Y. Kim, Roddy S. O'Connor, Stephen R. Wallace, Miroslaw S. Kozlowski, Dylan M. Marchione, Maksim Shestov, Benjamin A. Garcia, Carl H. June and Saar Gill; Nat Biotechnol (2020); 38(8):947-953

Intellectual property

Learn about the challenges of patenting CAR-T products

DISRUPTIVE CAR-T THERAPY IS DRIVING A NEW WAVE OF PATENTING CHALLENGES

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Immunotherapies, including monoclonal antibodies and checkpoint inhibitors, are a hugely promising therapeutic area, in terms of both clinical benefits and potential market size. These treatments are able to reactivate the body's own immune response to increase effectiveness in fighting diseases, particularly cancers. Among immunotherapies "adoptive immunotherapies" such as CAR-T cells (T cells engineered with chimeric-antigen-receptors (CARs)) are probably the highest hopes for patients and turn out to be a hot topic for healthcare and pharmaceutical industries. CAR-T therapy is indeed a revolutionary type of treatment which has emerged as a potent new class of therapeutics for cancer, based notably on their remarkable potency in blood cancers.

INTRODUCTION

CARs were first described more than 30 years ago. But only over the last decade has biomedical research really focused on the potential of CAR-T cells to create medicines, with an ever increasing clinical trials in progress. Three CAR-T treatments have now been approved: Kymriah® (tisagenlecleucel) was first to receive FDA and EMA approbation, followed a few months later by Yescarta® (axicabtagene ciloleucel) and FDA approbation for Tecartus® (brexucabtagene autoleucel) at the end of July 2020. All of these CAR-T therapies are "autologous" therapies, based on using the patient's own T cells. The process takes several weeks and is extremely expensive.

However, new approaches entail new challenges. effective patent strategy requires careful planning, especially in view of how increasingly crowded the CAR-T field has become. Infringement scenarios are different from those that have been traditionally considered for patents for pharmaceutical products. Companies will have to devise early on a sound patent strategy and thoroughly vet their products and processes for any freedom-to-operate issues. This article reviews the different approaches to CART-T cells patents with a focus on the European perspective. While not looking for exhaustivity, it will illustrate the complex intellectual property issues which have to be faced when trying to protect and/or commercialise CAR-T cells in Europe.

About Regimbeau

REGIMBEAU
Creative IP

REGIMBEAU, a French IP law firm, has been assisting companies and private and public project developers to protect, enhance, and defend their innovations and creations (patents, trademarks, designs) for more than 85 years. Fifteen partners head a team of more than 200 people whose skills are put into practice in every strategic aspect of Intellectual Property – business intelligence and information search, license agreements, IP portfolio audits, partnership negotiations, acquisition of industrial property rights, litigation. A dedicated team of technical and legal experts, with hands-on experience in tackling issues and challenges of innovation in immunology, can assist you in protecting your inventions with your best interest in mind. More info on our specific **webpage**.

CAR-T CELLS AND THEIR THERAPEUTIC USES

CAR-T cells therapy is based on *in vitro* engineering T-cells so that they express artificial receptors (called a "chimeric antigen receptor (CAR)") on the surface, thereby directing the CAR-T cells to specifically bind cancer cells.
CARs are synthetic proteins built by connecting several functional parts from different proteins, each with a specific function. A single-chain antibody variable fragment (scFv) recognises a specific protein on the surface of the malignant cells (e.g., CD19 on B-cells), thereby targeting the tumour. This antibody portion is connected via a flexible linker to a transmembrane segment which anchors the CAR at the surface of the T cell. Inside the cell, one or more domains are taken from signalling proteins, that ensures the T-cell receptor (TCR) signalling necessary to activate the effector functions of the CAR T-cell once it finds a tumour cell (cf. Figure 1).

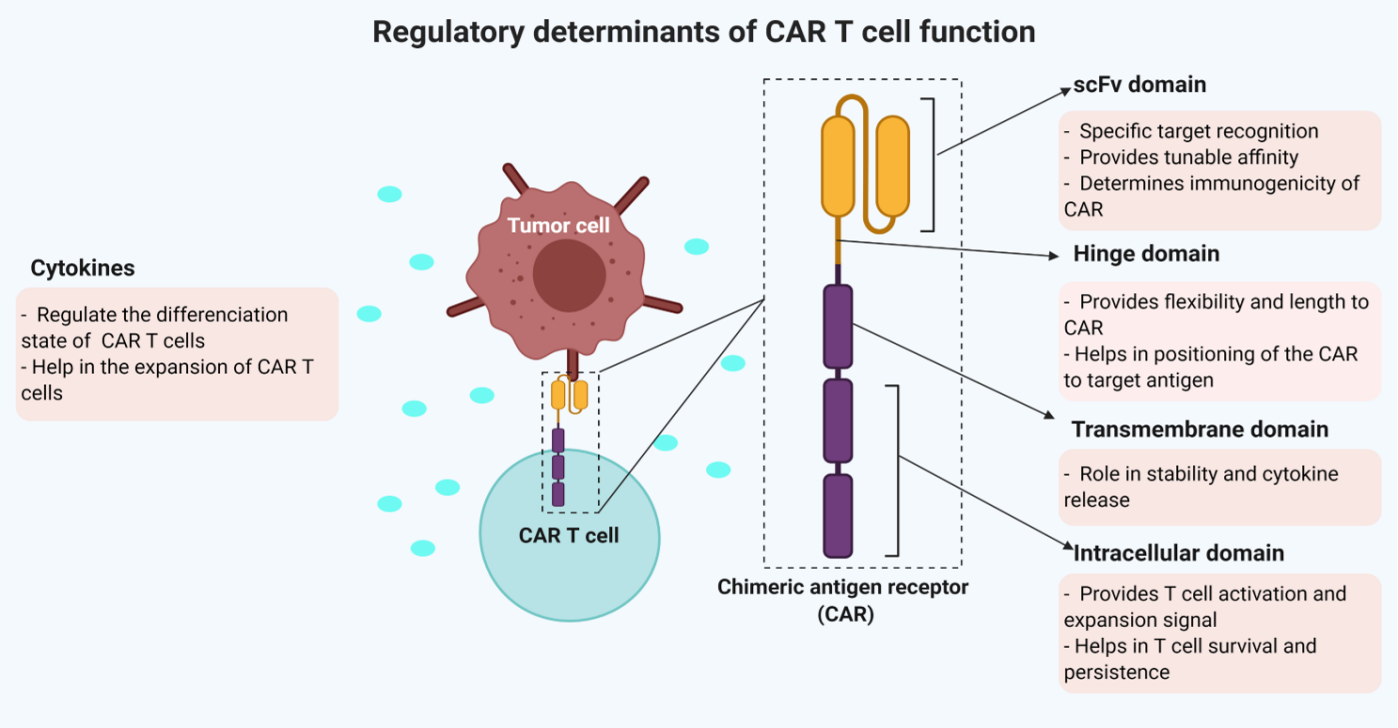

Figure 1: Structure of a chimeric antigen receptor (CAR)

In CAR-T cells therapy, individual patient's cells are collected, genetically modified, and returned to patient with new cancer-fighting properties. The various stages of this therapy are shown in Figure 2.

First, immune T cells are extracted from the patient's blood by leukapheresis. Then, the patient's T-cells are sent to the lab, where they are transfected with a recombinant vector encoding the CAR receptor, thereby making them CAR T-cells. Transfected T-cells are expanded *ex vivo* in platforms controlled for all aspects of safety and reproducibility in order to obtain enough cells for treating the patient. The cells are re-administered to the patient intravenously after chemotherapy conditioning.
The need to collect T-cells, to carry out the cell engineering and growing the genetically modified T cells, and then to return them into the patient's body, makes such a therapeutic process complicated and expensive. Collecting the T-cells from the patients and infusing back the engineered T-cells to them being performed at hospitals whereas the genetic modifications of the cells to produce CARs on their surface

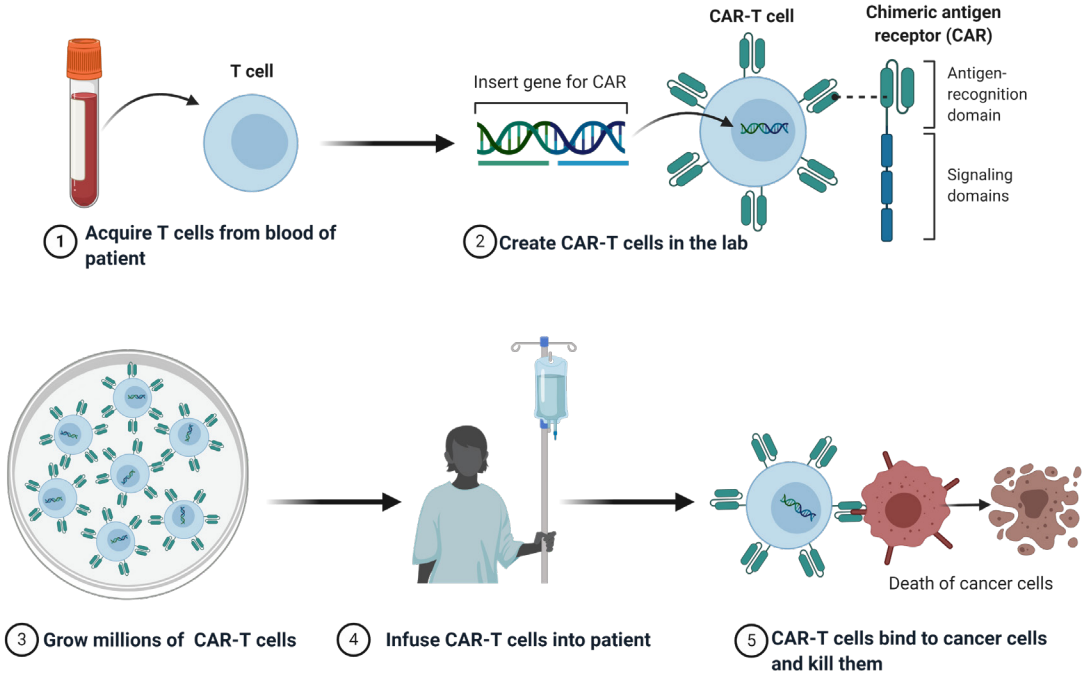

Figure 2: CAR-T Cell Therapy

is carried out by pharmaceutical industry. The key difference between conventional drugs and CAR-T cells therapies is that in the latter, each patient receives a treatment engineered specifically for them using their own immune cells, generating ambiguity around what the "drug" really is.

Due to these particularities of the treatment process, finding the more appropriate and effective patenting strategy is a real challenge, notably since this process may involve different steps being performed by distinct entities, which may furthermore not be located in the same country.

A DENSE INDUSTRIAL PROPERTY SITUATION

It is a sprint for pharmaceutical companies who are entering or continuing to do research into the development of the most optimal CAR-T cell therapy, manufacturing process and delivery method. The more patents a single pharmaceutical company can be issued that relate to CAR-T cell therapy; the greater market control a company will retain in this immunotherapy field.

As with many new biomedical developments, the firms bringing CAR-T treatments to market have filed numerous patent applications covering various aspects of these treatments. Different companies have their own proprietary CARs, with modifications in the transmembrane and endodomain co-stimulatory structures and signal peptides, in addition to the antigen recognition regions.

In April 2019, Björn Jürgens and Nigel Clarke published a study of CAR-T patenting commissioned by the European Patent Office (EPO). They found that it was in 2013 that the patenting of CAR-T inventions began in earnest with sixty filings around the world; it then increased through 2016 to 597 filings. When analysing countries by their number of CAR-T cell applicants, they found that US and China had the most applicants, with 39% and 33% respectively, followed by the UK (5%), Germany (5%), Japan (4%) and France (3%). As expected, the U.S. and China were the most productive countries, followed by the Switzerland, the U.K., Germany, and France. Parties looking today to protect their CAR-T inventions thus have to make with a very crowded patent field. As additional companies

[1] http://documents.epo.org/projects/babylon/eponet.nsf/0/5BE8186BE71C52FCC12584C100498651/$File/patent_insight_report-chimeric_antigen_receptor_t-cell_immunotherapy_en.pdf

proceed with CAR-T cell research, it is likely one will observe a steady increase in similarities between patent applications. This will inevitably lead to narrower claims being granted (if any) in many cases. However, in any case, a precise freedom-to-operate analysis cannot be omitted to fully and safely drive commercialisation of each specific CAR-T cells therapy.
Above all, the quest for the most appropriate patent protection for such an uncommon and disruptive therapies increasingly requests conceiving and designing new, imaginative patent strategies.

PROTECTING CAR-T CELL PRODUCTS

Patent laws around the world grant patents for two broad types of inventions: inventions relating to products (or "composition of matter") and inventions relating to methods. Product claims offer the broadest protection and are thus the most valued. They provide to the pharmaceutical companies a full ownership on the compound which is commercialised, whatever the uses thereof. Product claims should therefore be sought in any patent strategy for protecting CAR-T inventions.
For example, patent protection could also be sought for the CAR-T-cells themselves. However, this strategy is complicated by the very nature of the product. The fact that the treatment is unique and specific to each patient makes it difficult to define structurally. Obtaining a patent directed to CAR-T cells will probably be a long and arduous process. Moreover, even if this patent is granted, the real challenge will arise at the point of enforcement. CAR-T cells are individually produced for each patient. Therefore, infringement would, in principle, need to be established on a patient-by-patient basis. As a result, proving infringement will likely be complex. If testing is needed to check whether they fall under the claims in the patent, that will cause difficulties not encountered with mass-produced medicines. Another challenge will reside in the determination of the infringing sales, which are in turn used by the courts for setting the damage. And of course, preliminary injunction is out of the picture, since lives are directly at stake.
Alternatively, protection could be sought for specific CAR construct designs or their component parts. An example of such a claim is represented by claim 1 of European Patent EP2 935 321:

> *"1. A polypeptide comprising (i) a transmembrane domain from CTLA4 or PD-1, (ii) a CD3ζ intracellular signaling domain, and (iii) an extracellular domain that binds to an antigen on a tumor cell, wherein said polypeptide is a chimeric antigen receptor (CAR) and a T lymphocyte expressing said polypeptide is activated or stimulated to proliferate when said polypeptide binds to said antigen, and wherein if the transmembrane domain is from CTLA4, the extracellular domain of said polypeptide is not from CTLA4; and if the transmembrane domain is from PD-1, the extracellular domain of said polypeptide is not from PD-1."*

Many patent applications are thus directed to particular CAR constructs, and/or CAR-encoding polynucleotides. Such claims are highly valuable, as shown in a recent case in which Kite Pharma's Yescarta was found to infringe Juno Therapeutics' patent directed to a CAR-encoding polynucleotide. However, it is increasingly difficult to obtain broad claims in view of the accrual of publications and patent applications in the field. Even protecting a specific CAR construct may be challenging if this receptor does not possess an unexpected property, i.e. a feature which distinguishes this CAR from the prior art and could not have been predicted from said art.
This is because, oftentimes, the claimed construct will be built with the same basic domains (an scFv, a flexible linker, a transmembrane domain, a signalisation module) in the same arrangement, as CAR of the prior art. Furthermore, the claimed

[2] Juno Therapeutics, Inc. et al. v. Kite Pharma, Inc., No. 2-17-cv-07639 (C.D. Cal.).

construct's target will most often be a protein already known and characterised in the art. In such situations, the EPO will conclude that, absent an unexpected property, it is obvious to combine and try all these different protein sequences.
This objection can be overcome if the claimed construct possesses an advantage over the prior art which could not be foreseen. Note that it must be at least plausible that the claimed receptor does indeed possess this property. As of now, each CAR construct needs empirical testing for evaluation; indeed, small modifications can have major consequences on the therapeutic outcome. However, supporting experimental data will be needed to convince the examiner. Thus, one major focus for patenting activity is the development of new CARs with new characteristics. Among these features CAR-T cells with modified or added intracellular components, which result in improved or altered biological activities, including better activation of the CAR-T cells in response to target binding together with bispecific targeting strategies, and an improved capacity to kill target cells are of a significant interest. Besides, mitigating the off-target toxicities of CAR-T cells involves a variety of mechanisms to be discovered but which are essential for maintaining the brilliant potentials for CAR-T cells therapy and thus potentially represents a new avenue for patenting. Moreover, applying CARs to the treatment of solid cancers is also among the exciting new advancements which could significantly improve the patentability of a CAR.

POLYNUCLEOTIDES AND PROTECTING CAR-T CELL PRODUCTS

The CARs and their components may also be protected by virtue of the genetic sequences encoding these polypeptides. Actually, with infringement in mind, one may conclude that polynucleotides claims have a greater utility than claims directed to polypeptides. Proving infringement of a polypeptide claim will present a challenge, since the CAR is only expressed in the patient's cells. The nucleic acid, on the hand, may be prepared and purified in bulk before transfection, like any small molecule or biotech product. A patentee will thus be able to establish infringement for that polynucleotide generally. In this regard, it is worth noting that the claims found to be infringed in a California court by Kite Pharma (Gilead) were directed to nucleic acids encoding CARs :

> *"1. A nucleic acid polymer encoding a chimeric T cell receptor, said chimeric T cell receptor comprising (a) a zeta chain portion comprising the intracellular domain of human CD3 ζ chain, (b) a costimulatory signaling region, and (c) a binding element that specifically interacts with a selected target, wherein the costimulatory signaling region comprises the amino acid sequence encoded by SEQ ID NO:6."*

PROTECTING METHODS USING CAR-T CELLS

Although product claims have traditionally been a priority for pharmaceutical companies in terms of patent strategy, obtaining and/or enforcing such claims in relation to CAR-T inventions may probably be difficult as detailed above. At least for these reasons, applicants may want to consider method claims, as they have an increasing significant value, even more so than product claims, for protecting these personalised medicines.

In particular, patent claims directed to methods of manufacturing CAR-T-cells may be particularly valuable. This is because the same process for producing a specific CAR-T cell is performed for each patient, even though the resulting product is personalised for the specific patient. Methods of manufacturing are generally eligible for patent protection in most jurisdictions throughout the world. Any process by which the manufacturing process can be made more efficient, make a

[3] Board of Appeal decision T 1329/04
[4] U.S. Patent No. 7,446,190

better product or do it more cheaply is likely to provide competitive advantages. Claims covering all or part of a process that becomes a *de facto* standard required by the health authorities would be especially valuable.
An example of a manufacturing claims is represented by claim 1 of European Patent EP 3 134 095:

> *"An in vitro method for manufacturing a T cell therapeutic comprising: a) providing a population of human peripheral blood mononuclear cells (PBMCs) that comprises T cells and antigen presenting cells (APCs); b) culturing the population of PBMCs for 16 hours to 32 hours prior to transduction in a cell culture medium comprising i) interleukin-2 (IL-2), ii) an anti-CD3 antibody or CD3-binding fragment thereof, and iii) an anti-CD28 antibody or a CD28-binding fragment thereof, B7-1 or a CD28-binding fragment thereof, or B7-2 or a CD28-binding fragment thereof, wherein the culture activates and stimulates the T cells; c) transducing the population of PBMCs activated in step b) with a lentiviral vector encoding a chimeric antigen receptor (CAR); and d) culturing the population of PBMCs in a cell growth medium to expand the transduced T cells; thereby manufacturing the T cell therapeutic."*

Claims directed to methods of manufacturing face challenges, however, that product claims do not. First, they cover the commercialised CAR-T product only inasmuch as it is produced by the claimed process. If a competitor manages to make the same CAR-T cell by a different process, then it will avoid infringing the claims of the method patent. Further, the manufacturing process of CAR-T cells involve different actors, such as e.g. the medical practitioners who collect the patient's T cells and inject the CAR-T cells, the manufacturing plant where the vector carrying the CAR gene is produced and purified at a pharmaceutical grade, the facility wherein the T cells are transfected by the vector, etc. It is probable that not all of these actors are under the same control. The most useful claims will have to define methods in such a way that all steps will be performed by the same party (i.e. there is a direct infringement).
A further level of complication is reached when considering that steps relating to manipulation and processing of the cells are likely to take place in a number of different countries, particularly as manufacturing processes are scaled up. Successful enforcement of the patent may then depend on the jurisdiction. Demonstrating that infringing processes are being carried out in other jurisdictions is a potentially difficult and costly exercise. When drafting and prosecuting claims for CAR-T therapies, applicants should therefore consider carefully the practicalities of how and where steps of the therapeutic process will be performed.
CAR-T developers should also consider pursuing claims directed to methods of treatment with the CAR-T cells. These claims are allowed, in one format or the other, in the most commercially-important jurisdictions. They are particularly useful against manufacturers of generics and biosimilars, because these companies sell copies of the commercialised CAR-T product and in the same indication as authorised by the relevant health agencies. On the other hand, their scope is narrow. Hence, if the claimed medical use does not encompass the authorised indication, difficulties in enforcing these claims will arise. In addition, it will be difficult to use such claims against an originator developing a related CAR-T product in a different medical indication.

FREEDOM-TO-OPERATE

It is important for actors willing to develop a new CAR-T therapy to mitigate the risk of future infringement litigations by assessing their freedom-to-operate with respect to third-party patents and patents that may issue from pending applications. That a therapy is personalised does not mean it is free of rights from third parties. In fact, the number of patents involving CAR-T therapy, covering all aspects from the subdomains of receptors to dosage regimen of specific CAR-T cells, is already staggering and continues to grow. Moreover, even if an infringement suit is not successful, it may be extremely costly, thereby draining vital financial resources away from ongoing research projects. It makes the question of freedom-to-operate more pressing.

The earliest CAR-T patents are the broadest and should be monitored carefully. But that does not mean that more recent patents, especially those covering manufacturing methods or medical uses, should be ignored. It must also be emphasised that CAR-T inventions cover several aspects, each of which may be protected independently of its role in a CAR-T context. For example, each of the constituents of the receptor may be covered independently, so that a specific evaluation must be carried out for each of them. Likewise, it should be cautiously checked for each step of the manufacturing method if it falls in the scope of a third-party patent. Specific medical uses should also be evaluated for their freedom-to-operate, including such aspects not directly related to CAR-T as preconditioning of patients (before administration of CAR-T cells). Therefore, any comprehensive freedom-to-operate analysis should include an evaluation of patents directed to these various, more general aspects of CAR-T therapy.

Of course, freedom-to-operate assessment of a new CAR-T invention will significantly add to the overall costs of the whole project. However, when considering the risks of not doing so, verifying that all aspects of the invention are free from third-party patents appears to be money well spent. Failure to properly check that an aspect of a CAR-T invention is free may result in a highly relevant patent not being identified and facing a litigation suit later on, once the therapy is on the market. The backlash then will most probably be severe: in the Yescarta infringement suit, Juno Therapeutics (BMS) was awarded 1.1 billion dollars in damages from Kite Pharma (Gilead).

CONCLUSIONS

Investments and researches in the CAR-T therapy field are continuously growing; however, with the numerous technical challenges, significant challenges in obtaining appropriate patent protection remain.

Due to the highly personalised approach and very specific targeting issues, CAR-T cells cannot be manufactured by the drug development path as usually practiced in the pharmaceutical field. The way by which the pharmaceutical companies will protect their investments through patenting must be adapted. As it does not fit into the traditional model of patentable therapy, it is indeed necessary to explore new strategies.

Still, infringement circumstances are also different to those that have been traditionally considered for patents in the pharmaceutical field. With such innovative and huge therapeutic properties associated with a growing number of patents and applications in this field, a large amount of patent litigation is expected in the years to come.

Conversely, as with any nascent technology, research is intensively underway, especially to discover how to expand such therapy to additional patients and offer valuable patenting opportunities. For example, as the CAR-T therapies approved up to now are “autologous” therapies, i.e. based on using the patient’s own T cells, the process takes several weeks and is extremely expensive. Researchers are already working on “off-the-shelf” or “allogeneic” products, that use donor CAR-T cells and would allow the treatments to be made available more quickly and cheaply. Allogeneic CAR-T cells procedures could thus provide significant advantages in manufacturing, including reducing costs and simplifying the supply chain.

With this way of scientific advancements, which in a sense un-personalised such therapy, patenting approaches could come back a few closer to those traditionally practiced. Only innovative future will let us know but patenting experts are for sure ready in the starting line!

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Raphaëlle GILLET is a French and European Patent Attorney. She has a Ph.D. in Molecular and Cellular Biology (Institut Cochin de Génétique Moléculaire, Paris), a MS in Cellular and Molecular Biology Development (Hôpital Necker, Paris) and she has a CEIPI Graduate (Distinction in Patents and Trademarks). She started her career in Industrial Property in 2001. After an initial experience in a biopharmaceuticals start-up, followed by twelve years' experience in an IP Law firm, Raphaëlle joined **REGIMBEAU** in 2014. She assists her clients and supports them in the development, management and defense of their portfolio. Raphaëlle also provides seminars and courses on Intellectual property, in order to make IP accessible to all.

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CAR-T patents

Even though the chimeric antigen receptor field can be considered as new when compared to other types of biologics, the patent landscape around this particular technology is already significant. Indeed, our **BI&IP department at MabDesign** has identified at least **2402 patent families** so far* of which the majority being linked to CART. We here provide a sample of the different patents filed in 2020.

Month of application	Title	Patent number	Assignee
July 2020	Anti-CLEC 14A chimeric antigen receptor, T cell modified by same and application	CN111732666	Jinan Xingyi Medical Technology
	Anti-human MSLN antibodies and MSLN-targeted immune effector cells	CN111560072	Shanghai Hengrun Dasheng Biotechnology
	Chimeric antigen receptor targeting c-Met and autocrine PD-L1scFv and application thereof	CN111704674	Nanjing Medical University
June 2020	CAR-T technology for presenting and activating HSV-1 type oncolytic virus and application thereof	CN111676199	Wuahn Bio Raid Biotechnology
	Engineered cells for adoptive cell therapy	US20200318070	Juno Therapeutics
	Armed chimeric antigen receptor cell of targeted coronavirus SPIKE, preparation method and application	CN111675765	Nanjing Kaedi Biotech
May 2020	Anti-mesothelin chimeric antigen receptor, expression gene, expression vector, T cell and application thereof	CN111548420	Pregene Biopharma
	CAR-T cell for resisting novel coronavirus S protein, preparation method and application thereof	CN111518773	Jinan Xingyi Medical Technology
	Method to Treat Cancer with Engineered T-Cells	US20200297769	Lentigen Technology

* keyword search for 'chimeric antigen receptor' and associated terms in independent claims, title or abstract in the Orbit database on 12/10/2020

Month of application	Title	Patent number	Assignee
April 2020	Method for enhancing cellular immuno-therapy	WO2020/206395	Fred Hutchinson Cancer Research Center Nektar Therapeutics
	Manufacturing anti-bcma car t cells	WO2020/206061	Bluebird Bio
	Dual Antigen-recognizing iPS cell-derive chimera antigen receptor-expressing T-cell therapeutic method	WO2020/204086	Juntendo Educational Foundation University of Tokyo
March 2020	Genetically reprogrammed tregs expres-sing cars	WO2020/194306	Gavish Galilee Bio Applications
	Car-t cell therapies with enhanced efficacy	WO2020/191316	Novartis University of Pennsylvania
	Improved process for integration of dna constructs using rna-guided endo-nucleases	WO2020/185867	Sorrento Therapeutics
February 2020	High affinity engineered t-cell receptors targeting cmv infected cells	WO2020/167957	University of texas
	Treatment involving car-engineered t cells and cytokines	WO2020/161224	Biontech Cell and Gene Therapies
	Car library and production method for scfv	WO2020/162452	Ehime University
January 2020	Signaling platforms for chimeric antigen receptor t cells	WO2020/160419	DartMouth College
	Humanized bcma antibody and bcma-car-t cells	WO2020/150339	Caribou Biosciences
	Car t cell methods and constructs	WO2020/142780	Tocagen

Patents filed in 2020 (87 in total) were sorted one by one to check for pertinence to CART.
A sample of 3 patents filed per month was randomly selected.

For more information about our IP services and patent search strategy, please contact our BI&IP department.

Training

Explore your new section dedicated to skill development : CAR-T cell training, partner news and funding opportunities

Training and CAR T cells, a novel story…

Skill development is an essential building block to ensure that scientific projects are completed successfully and for innovative companies to thrive. Despite the current explosion of CAR T-cell R&D in France and all around the world as evidenced by the information presented previously in this edition, our training department at MabDesign have however identified a surprising low number of training programs dedicated to this topic.

In order to identify the training axises to be developed, an internal study has led us to define six challenges for the research, development and industrialization of CAR-T cells :

Different cellular types and therapeutic strategies: T-cell, NK cell, IPS…

Target selection: safety and toxicity associated

CAR engineering for solid tumor

CMC: accessibility and availability of raw materials

Regulatory affairs

Autologous cell therapies: bioprocessing and yield increase

Based on our expertise in the field of advanced therapy medicinal products, which include CART technology, we are convinced that the mastering of these specific topics is essential to resolve the current limitations and avoid the pitfalls in CAR T-cell technologies. The creation of new training programs on CAR-T by MabDesign should integrate these strategic dimensions.

Discovering the amazing world of CAR T cell therapy with ENSTBB!

It is not an understatement to say that CAR T cell therapies have been one of the major breakthroughs of recent years in the Health Sector. This technology represents a huge hope for patients suffering from incurable rare diseases or cancer, as well as a very dynamic driver of economic growth for pharmaceutical firms. A number of companies, from small Biotech firms to Big Pharma, have already joined the crazy race towards CAR T cell- based therapies, using various strategies and at different stages of the value chain (antigen targeting, CAR design, side effects, industrial production, drug delivery and patient monitoring). Today, the challenge is both to validate scientific concepts and to industrialize the production process by making it more qualitative, more efficient and above all safer for patients at a reasonable cost. And despite the wonderful promise of the first two products on the market (three products in the US), and the hundreds under development, a huge amount of work still needs to be done.

Another important challenge concerns knowledge and training: it is not always easy for non-specialists to find an appropriate level of information or to select the relevant data in the jungle of publications. Furthermore, there are few university courses dealing with CAR T cells today, and the existing ones are quite recent, so professionals from pharma or biotech companies who have turned to this field often have a lack of academic background on these topics. The need is real for these persons, who usually have a pharmaceutical, biological or chemical background, to be trained in these concepts, but the choice of courses is limited for non-experts, even in continuing education.

That was the reason why, in 2019, the ENSTBB continuing education team created a course called “Discovering the principles and issues of CAR T cells in immunotherapy” for the 2nd edition of “Master Classes of Immunotherapy” co-organised by the ENSTBB and MabDesign.
This course targeted professionals without any knowledge in cell and gene therapy. Its purpose was to give the tools and expertise necessary to understand the principles and issues at stake in order to work properly with experts of the field. It was custom-built to help participants apprehend the vocabulary and fundamental concepts used in adoptive T-cell therapy. During the course, participants were taught to understand the mechanisms of action of CAR T cells in oncology, to identify the main steps necessary for the production of CAR T cells, and to grasp the main issues and future trends related to therapy by CAR T cells (toxicity, application to solid tumors, allogeneic cells...).

Bordeaux INP
ENSTBB

HIGHLIGHTS OF THE LATEST MASTER CLASSES OF IMMUNOTHERAPY EDITION

MASTER CLASSES 2020 DE L'IMMUNOTHÉRAPIE

3e édition - 26 et 27 octobre 2020
100% distanciel

7 formations en parallèle :

Evalutation de la développabilité des biomédicaments
Impuretés e contaminants en bioproduction
Intensification des procédés
Immunologie et immunothérapie
Principes et enjeux des CAR-T cells
Génération et ingéniereie des anticorps
Développement clinique des anticorps

4 webinars technologiques :

Bioluminescence / Cell line development
AlphaLISA et HTRF / Méthodes moléculaires en analytique

56 personnes formées
+ de 100 personnes réunies en formations et en webinars
Cette édition des Master Classes de l'immunothérapie a obtenu le score de 90 % de satisfaction

Partenaires réseau

Les dispositifs de financement à disposition des entreprises

Cet article est issu du site web de l'OPCO 2i, avec l'accord de la direction de l'OPCO 2i Auvergne-Rhône-Alpes.

Depuis le 1er janvier 2020, OPCO 2i est le nouvel opérateur de compétences et formation des entreprises du secteur industriel (dont pharmaceutique, et activités de recherche et développement en biotechnologies). Les missions de l'OPCO 2i sont d'informer, de conseiller et d'accompagner les entreprises dans la mise en œuvre des projets RH, compétences, formation et apprentissage.

Réforme 2018 : pourquoi des opérateurs de compétences ?

La loi « Pour la liberté de choisir son avenir professionnel », adoptée le 5 septembre 2018, a modifié profondément le système de la formation professionnelle tant au niveau des dispositifs que des financements et des acteurs.
En créant les opérateurs de compétences, l'objectif est de permettre aux branches professionnelles de se réunir dans une logique de filière pour créer des ensembles cohérents en termes de métiers, de compétences, de mobilité ou encore d'enjeux liés à la formation.
Depuis le 1er avril 2019, 11 opérateurs de compétences (OPCO), dans lesquels sont réparties 329 branches professionnelles, ont remplacé les 20 opérateurs paritaires collecteurs agréés (OPCA). C'est dans ce contexte qu'a été signé l'accord portant création d'OPCO 2i. Ce nouvel opérateur de compétences interindustriel rassemble 32 branches professionnelles, dont les branches de l'industrie pharmaceutique et des activités de recherche et développement en biotechnologies. Il reprend partiellement ou totalement les champs de compétences des OPCA OPCAIM, OPCA DEFi, OPCA 3+, AGEFOS-PME et OPCALIA.

Financer vos projets de formation

L'optimisation des dépenses formation des entreprises et la recherche de financement est le cœur de métier de l'OPCO2i. Plusieurs dispositifs sur fonds mutualisés sont à la disposition de la filière, que ce soit dans le cadre du plan de développement des compétences pour une entreprise de -50 salariés ou pour les dispositifs alternance.

En mutualisant les fonds de la formation professionnelle et de l'apprentissage, OPCO 2i finance les actions de formation sur plusieurs dispositifs :

- Diagnostic GPEC, thématique et accompagnement RH
- Plan de développement de compétences (-50 salariés)
- Préparation opérationnelle à l'emploi
- Contrat de professionnalisation
- Contrat de professionnalisation expérimental
- Formation de tuteur et fonction tutorale
- Contrat d'apprentissage
- Formation et fonction maitre d'apprentissage

Plan de développement des compétences des entreprises de – de 50 salariés

Prise en charge forfaitaire :

- Prise en charge au coût réel plafonné à **30 € / heure** pour toutes les formations (cœur de métier ou autres formation)
- Prise en charge de la rémunération pour toutes les entreprises de moins de 50 salariés dans la limite du SMIC brut (**10,15 € / heure**)
- **Suppression du plafond** annuel de 6 000 € / entreprise
- Les **actions « clé en main »** sont également prises en charge au coût réel plafonné à 30 € HT/ heure

Contrats d'apprentissage

Ce dispositif s'applique à toute taille d'entreprise.

- Les niveaux de prise en charge définis par les branches et validées par France compétences s'appliquent. Ils sont à télécharger sur le site de **France compétences** (www.francecompetences.fr)
- Majoration de 50 % du niveau de prise en charge dès lors que le bénéficiaire du contrat d'apprentissage est reconnu en situation de handicap

Forfait soutien à la mobilité internationale et européenne pour les jeunes apprentis

- *Fonction de référent mobilité internationale dans le CFA : forfait de 500 € par apprenti en mobilité.*
- *Réel plafonné dans la limite de 800 €/an par apprenti (avec THR)*

1er équipement de l'apprenti

- *Forfait de 500€ pour les formations industrielles*
- *L'équipement numérique sous certaines conditions (néanmoins non précisées)*
- *Les EPI, notamment l'équipement lié aux mesures sanitaires COVID-19*

Frais d'hébergement de l'apprenti :

- *Hébergement : **6€ /nuitée**, petit déjeuner inclus*

Frais de restauration de l'apprenti :

- *Repas : **3€** dans la limite de 2 repas / jour*

Exercice de la fonction tutorale et formation des maîtres d'apprentissage

- ***230€ par mois** et par apprenti dans la limite de 12 mois et prise en charge de la formation des maîtres d'apprentissage dans la limite de 40h et de 15€/h.*

Validation des acquis et de l'expérience (VAE)

- Forfait accompagnement 1500 €

List of abbreviations and acronyms

CAR: Chimeric antigen receptor
CD: Cluster of differentiation
CLL: chronic lymphocytic leukemia
CR: Complete remission
CRS: cytokine release syndrome
CTU: cell therapy unit
B-ALL: acute lymphoblastic leukemia
BCMA: B-cell maturation antigen
BCP-ALL: B-cell precursor acute lymphoblastic leukemia
EMA: European Medicines Agency
EPO: European Patent Office
EU: European Union
FDA: Food and Drug Administration
GVHD: graft-versus-host disease
HSPG: heparan sulfate proteoglycans
HLA: human leukocyte antigen
HSCT: Haematopoietic stem cell transplantation
ICANS: immune effector cell-associated neurotoxicity syndrome
IL: Interleukin
iNKT: invariant Natural Killer T cell
NA: Not applicable
NCR: natural cytotoxicity receptors
NFAT: nuclear factor of the activated T cell
NK: Natural Killer cell
NKT: Natural Killer T cell
NHL: Non-Hodgkin's lymphoma
MCL: Mantle Cell Lymphoma
MM: multiple myeloma
MRD: Minimal residual disease
PBMC: Peripheral blood mononuclear cells
PRIME: Priority Medicines
R/R: relapse/refractory
scFv: single chain variable fragment
TCR: T cell receptor
TRUCKS: T cells redirected for antigen-unrestricted cytokine-initiated killing
UK: United Kingdom
US: United States

UPCOMING MABDESIGN EVENTS

Join us at our next scientific and networking events in 2020

400+ Attendees

40+ High-profile speakers

180+ Different organizations present

40 Exhibitors

450 B2B meetings taking place

8TH AIS 2020

Multispecific Antibodies, Ab-like Molecules & Adoptive Cell Therapy for Fighting Diseases

NOVEMBER 23-24, 2020

100% DIGITAL

MAbimprove LabEx Tours Montpellier

MABDESIGN THE IMMUNOTHERAPY NETWORK

Keynote speakers

Michael Hudecek
Universitätsklinikum Würzburg, Germany

Dario Neri
Philogen & ETH Zurich, Switzerland

Christian Klein
Roche, Switzerland

Paul W. Parren
Lava Therapeutics, Netherlands

immunotherapies & innovations for Infectious Diseases

4th Congress 2020 – 9 & 10 December 2020 - Digital format

SAVE THE DATE

Key figures from the previous edition...

- 350+ Attendees
- 40+ High-profile speakers
- 150+ Different organizations present
- 200+ B2B meetings taking place

MABDESIGN THE IMMUNOTHERAPY NETWORK

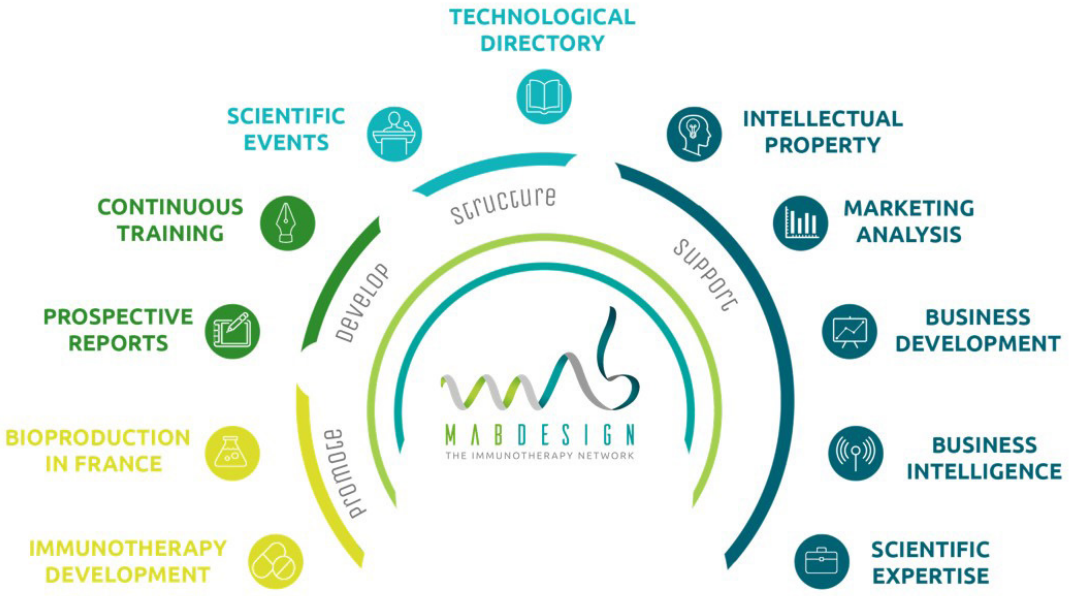
BIOASTER MICROBIOLOGY TECHNOLOGY INSTITUTE

LYONBIOPOLE Auvergne - Rhône - Alpes

www.i4id.org

About MabDesign

- **MABDESIGN** is a French membership organization in the field of immunotherapy. Created in 2014 MABDESIGN is managed by four competitivity clusters (Atlanpole Biotherapies, Eurobiomed, Lyonbiopole, Medicen) and two pharmaceutical companies (Pierre Fabre and Sanofi), and one biotech (DBV Technologies).



- **Operational since September 2015**, MABDESIGN has over 170 members, including pharmaceutical and biotechnology companies, service providers, training organizations, and equipment suppliers at the cutting edge of technology.

Next on Immunowatch

- **Bioprocessing**

Acknowledgement: Several figures presented in the document were generated using the Biorender online plateform.

MABDESIGN
THE IMMUNOTHERAPY NETWORK

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