CAR-T – The Future Promising Therapy for CLL: A Mix between Cell Therapy, Gene Therapy and Immune-Oncology

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Abstract

Chronic lymphocytic leukemia (CLL) has considerable change since the time clinical staging was introduced in clinical practice in 1975. Over the years, the prognostic factors analyses in CLL have expanded, and thus treatment considerably changed. Based on discovery of specific mutations, several targeted therapies have been introduced. This progress is continuing and finally chimeric antigen receptor against T cells (CART) is in the process of being developed. This review is an attempt to summarize the major benchmarks in the CART therapy of CLL.

Keywords: Chronic lymphocytic leukemia; CAR-T cell therapy; ROR1

Introduction

B-cell chronic lymphocytic leukemia (CLL) is the most common lymph proliferative disorder [1]. CLL is characterized by the clonal expansion of mature antigen-stimulated B-cells (CD10+/CD5+/CD23+) in the blood, secondary lymphoid tissues, and bone marrow (BM), in close contact with stromal microenvironment [1,2].

The 'traditional' way to treatment CLL is chemotherapy. One big problem with this technique is the lack of specificity as chemotherapies are targeting cancer cells, but also healthy cells. So, how to improve drug specificity? There are monoclonal antibodies (mAbs), which were discovered in 1975 in the UK [3]. Antibodies rapidly took off as a whole new approach for fighting cancer and leukemia. The first mAb used for cancer purpose was invented in 1983 to treat a patient with non-Hodgkin's lymphoma. Back then, having good results for the primary efficacy was not enough to negate all the adverse effects and limits of the therapy. However, since then the field has rapidly improved. Monoclonal antibodies are similar to chemotherapy in the respect they only act on a single target for example CD20 for Rituximab in CLL and B-cell lymphomas [4]. And because cancer cells are so versatile, as soon as one path is inhibited another one simply takes its place. To answer this problem, multitarget strategies have been developed instead, ex.bi-specific antibodies. On one hand, they block signal pathways, therefore killing the cancer cell. On the other hand, they are triggering the immune system to defend it against the cancer cells. Targeted tumor-specific cellular therapy will overcome many of the current limitations of adoptive immunotherapy.

How Does CAR-T Work?

The T cells play an important role in identifying and killing disease cells. Unfortunately, cancer cell develop mechanisms to evade immune system. Gene transfer techniques have now been developed to genetically modify T cells to confer novel antigen specificity by stably expressing, a chimeric antigen receptor (CAR) on their surface, providing them with the specific cancer targeting mechanism. CARs combine an antigen recognition domain of the CD3ζ (zeta) chain or FcγRI protein into a single chimeric protein [5]. When antigen is encountered, CAR-modified T cells become activated and kill in an antigen dependent, but HLA independent manner, making this an attractive approach as a generalized cancer therapy [6]. Adoptive cellular therapy involves the *ex vivo* enrichment and expansion of T lymphocytes. For therapies using T cells expressing transduced CARs or T-cell receptors, cGMP grade ancillary genetic modification reagents, such as retroviral and lentiviral vectors, are also required. One of the challenges of this largely personalized medicine is the development of efficient technologies and cost-effective clinical manufacturing platforms to support the later clinical trial phases and ultimately commercialization [7].

Adoptive cell therapy using CD19-targeted CAR T-cells has resulted in remarkable responses in adult patients with acute lymphoblastic leukemia (ALL) [8-11] as well as children [12,13]. CD19 is an ideal tumor target. Expression is restricted to B cells, from the pro-B cell stage to mature B cells (though not on plasma cells), possibly follicular dendritic cells, and it is expressed on the surface of most B cell malignancies. Importantly, it is not expressed on pluripotent marrow stem cells [14]. The initial success of CD19-targeted CAR-T cells in early phase clinical trials for the treatment of hematologic malignancies has triggered a genuine interest for CAR-T cell-based therapies among clinicians and researchers [8-11,15,16] as well as triggered active support and investments from pharmaceutical and biotechnology companies [17,18]. CAR-T123 might be also acute myeloid leukemia (AML) therapy and may be used as a novel-conditioning regimen for hematopoietic cell transplantation [19], anti-CD30 zeta artificial chimeric T-cell receptor has been used for immunotherapy of Hodgkin lymphoma (NCT01192464 and NCT01316146) [20], whereas CD19-targeted CAR-T was also very effective in treatment of NHL [21]. CD19, CD20 chimeric antigen receptor T (CAR T) cell therapy has shown promising results for allogeneic stem cell transplantation. Anwer at al. [22] has been reported best results in ALL patients with a complete response rate above 80%. A total of 72 patients from seven studies were treated with donor-derived CAR T cells. Only five out of 72 patients (6.9%) developed graft versus host disease [22]. These results are very promising.

The targeting of other types of cancers focusing on additional tumor-associated antigens, such as PSMA, mesothelin, GD2, HER2, and EGFR, is currently an active field of research and clinical trials as well [23]. Investigators used CAR-T cells to target hematological malignancies as well as solid tumors. The receptor tyrosine kinase human epidermal growth factor receptor 2 (HER2) is over expressed in multiple malignancies and has emerged as a logical target for adoptive cellular therapy for example in osteosarcoma [24], recurrent ovarian cancer [25], mesothelioma, pancreatic cancers (NCT02465983) [26], and glioblastoma multiforme (NCT02442297) [24].

Manufacturing of CAR-T cells

Despite the various designs and distinctive tumor-specific scFVs, the manufacturing procedure for CAR-T cells remains consistent. As a mostly autologous cell-based therapy, the CAR-T cell-manufacturing process starts from the collection of peripheral blood mononuclear cells from the patient, commonly achieved by a leukapheresis process. The patient should be in an appropriate window with the presence of sufficient numbers of T lymphocytes to collect. The most common subsets of T cells are CD4+, CD8+, CD25+ or CD62L+ [7]. In clinical trials are widely used CAR-T cells generated from CD3+ population [8-11]. Clinical selection, transduction and expansion processes have also been developed for these T-cell subsets [27,28].

Unlike autologous CAR-T immunotherapies that requiring individualized immunotherapy product for each patient the company Cellectis (France) produce allogeneic CAR-T immunotherapy, which is a non-patient specific or off-the-shelf product derived from a healthy T cell donor. To produce off-the-shelf T cells surface receptors on the cell must be modified. Within the cell TALEN gene editing is use to suppress specific surface receptors on T cells. Accustomed TALEN targets and binds to precise gene sequences. The DNA is clipped by TALEN resulting in a safer activation of a target gene. Through this gene editing the targeted receptors are removed

Figure 1: Chimeric antigen receptors (CAR), pioneered by Eshhar et al. 1989.
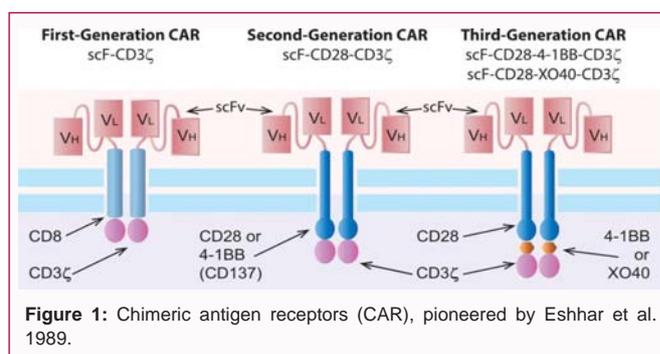
from the cell surface creating T cells with CAR proteins ready for use by patients. Allogeneic production of CAR T-cells is cost effective and results in a remarkable off-the-shelf product capable of being distributed worldwide. The new candidates UCART19 (universal CAR-T) with their proprietary TALEN gene-editing platform are used to target ALL and CLL [29].

CAR T-Cell Therapy for CLL

What does CAR-T look like?

There are differences between CART therapies. Some of them are effective in CLL some of them are not. First generation CAR (Chimeric Antigen Receptors) is T cell receptor that contains single chain fragment variable region of an antibody (scFv). First generation CARs use this scFv with the CD8 transmembrane protein and CD3ζ protein to help activating the downstream signaling (Figure 1). The first generation is really much less complicated but this is also less effective. So the cells within the attack their antigens, they do not have ability to sustain themselves or actually killed off the antigens. The number of co-stimulatory domains in this model is zero. In the huge number of different experiments is shown that are not in a co-stimulatory molecule either CD28 or 4-1BB (also known as CD137) cumulate dramatically improving the ability of the T cells to survive and target and kill the intended targets. The second generations CARs uses the same single chain fragments of the variable region and then use the CD28 or 4-1BB sections in a membrane and uses the CD3ζ chain as the intracellular signaling. In all clinical trials that are currently ongoing are really second generation CARs. The University of Pennsylvania (U Penn) uses a 4-1BB CAR, whereas University of California San Diego uses CD28 in a membrane. The main differences between those three CARs is that mainly is used CD28 than 4-1BB. To make a third generation CAR the additional unit 4-1BB or OX40 has been added to create highly functional CAR T. In literature there are two more described signaling domains such as those derived from ICOS [30] and CD134 [31,32]. The co-stimulatory molecules have different biological functions and thus may result in CAR T-cells of somewhat diverse functional capacities; for example CD28-costimulated CAR Ts result in initially potent effector functions, but the *in vivo* persistence of these cells appears to be inferior to that of CD137 (4-1BB)-co-stimulated T-cells [33]. The inclusion of the ICOS molecule appears to drive Th1/Th17 differentiation [30].

T-cell selection and activation

T-cell activation *ex vivo* requires a primary specific signal via the T-cell receptor (Signal 1) and co-stimulatory signals via molecules such as CD28, 4-1BB, or OX40 (Signal 2). Retroviral vectors are also needed for T cells activation required for the transduction of the CAR cDNA [7]. There are several methods of T cells activation. The simplest one is a cell-based T-cell activation. The endogenous

Table 1: Summary of the most recent clinical trials of CAR T-cell therapy in CLL.

Center	Biological	Status	Clin. trial.gov. No	Conditions	Phase	Title
MSKCC	EGFRt/19-28z/4-1BBL CAR T-cells	Recruiting	NCT03085173	CLL-Relapsed, Refractory	1	"Armored" CAR T Cells Targeting CD19 for Patients with Relapsed CLL
MDACC	ROR1-CART	Active not recruiting participants	NCT02194374	CLL, SLL	1	Autologus ROR1-CAR-T Cells for Chronic Lymphocytic Leukemia (CLL)
BCM	CD19 CAR T-cells	Recruiting	NCT01853631	CLL, NHL, ALL	1	Activated T-Cells Expressing 2nd or 3rd Generation CD19-Specific CAR, Advanced B-Cell NHL, ALL and CLL (SAGAN)
SG	Anti-CD19-CAR T-cells	Recruiting	NCT02672501	B-cell leukemia	1&2	A Study to Assess CD19-targeted Immunotherapy T Cells in Patients with Relapsed or Refractory CD19+ B Cell Leukemia
BCM	CD19-CAR-28-zeta T cells	Active not recruiting participants	NCT00586391	CLL, ALL, B-cell lymphoma	1	CD19 Chimeric Receptor Expressing T Lymphocytes In B-Cell Non-Hodgkin's Lympgima, ALL & CLL (CRETI-NH).
BDB	Anti-CD19-CAR T-cells	Active not recruiting participants	NCT02546739	Leukemia, Lymphoma	1	Immunotherapy with CD19 CART T-cell Lymphoma, ALL,CLL
BDB	CD19 CART γ δT-cell	Not yet recruiting	NCT02656147	Leukemia, Lymphoma	1	Immunotherapy with CD19 CART γ δT-cell for B-Cell Lymphoma, ALL and CLL
RH	CD19 CAR T-cells	Not yet recruiting	NCT02933775	Leukemia, Lymphoma	1	CD19-redirected Autologus Cells (CAR-CD19 T Cells)
FHCRC	Autologous Anti-CD19 CAR-4-1BB- CD3zeta-EGFRt-expressing T Lymphocytes	Recruiting	NCT01865617	CD19-Positive Neoplastic Cells Present	1&2	Laboratory Treated T Cells in Treating Patients with Relapsed or Refractory Chronic Lymphocytic Leukemia, Non-Hodgkin Lymphoma, or Acute Lymphoblastic Leukemia
CPGH	Anti-CD19/20-CAR vector-transduced T cells	Recruiting	NCT03097770	CD19 & CD20-Positive Neoplastic Cells Present	1	Treatment of Relapsed and/or Chemotherapy Refractory B-cell Malignancy by Tandem CAR T Cells Targeting CD19 and CD20
BCM	Autologous or syngeneic PBTLs and EBV-CTLs	Active not recruiting participants	NCT00709033	NHL, CLL	1	T-cells or EBV Specific CTLs, Advanced B-Cell NHL and CLL (ATECRAB)
BCM	CD19.CAR-CD28Z T Cells - dose escalation 1 and 2	Recruiting	NCT02050347	CLL, NHL, ALL	1	Activated T Lymphocytes Expressing CARs, Relapsed CD19+ Malignancies Post-Allo HSCT (CARPASCIO)
CPGH	Anti-CD19-CAR vector-transduced T cells	Recruiting	NCT01864889	CD19-Positive Neoplastic Cells Present	1	Treatment of Relapsed and/or Chemotherapy Refractory B-cell Malignancy by CART19 (CART19)
TFPHY	Prophylactic 4SCAR19 cells	Recruiting	NCT02968472	B-ALL	1	A Phase I Trial of 4SCAR19 Cells in the Treatment of Relapsed and Refractory B-Cell Leukemia
BCM	Kappa CD28 T cells	Recruiting	NCT00881920	Lymphoma, Myeloma, Leukemia	1	Kappa-CD28 T Lymphocytes, Chronic Lymphocytic Leukemia, B-cell Lymphoma or Multiple Myeloma, CHARKALL (CHARKALL)
HHUTCM	αCD19-TCRζ-CD28 and αCD19-TCRζ-CD137 CAR T-cells	Recruiting	NCT02685670	CD19+ B-lineage leukemia and lymphoma	1&2	Competitive Transfer of αCD19-TCRz-CD28 and αCD19-TCRz-CD137 CAR T Cells for B-cell Leukemia/Lymphoma (MatchCART)
FHCRC	ROR1CAR- specific Autologous T-Lymphocytes	Recruiting	NCT02706392	ROR1+ Malignancies	1	Genetically Modified T-Cell Therapy in Treating Patients With Advanced ROR1+ Malignancies
BCM	CD19 CAR/virus specific T cells	Active not recruiting participants	NCT00840853	CLL, ALL, NHL	1	Multi-virus CTLs Expressing CD19 Chimeric Receptors, CD19 Positive Malignancies Post SCT, MULTIPRAT
HSB	Autologous CD19-targeting CAR T-cells	Recruiting	NCT02963038	B cell leukemia, B cell lymphoma	1&2	CAR T Cells for Refractory B Cell Malignancy
UU	CAR T-cells	Recruiting	NCT03068416	B cell leukemia, B cell lymphoma	2	CD19-targeting, 3rd Generation, CAR T Cells for Refractory B Cells Malignancy
UU	Autologous 3rd generation CD19-targeting CAR T-cells	Recruiting	NCT02132624	B cell leukemia, B cell lymphoma	1&2	CD19-targeting 3rd Generation, CAR T Cells for Refractory B Cell Malignancy - a Phase I/IIa Trial.

MSKCC: Memorial Sloan Kettering Cancer Center; MDACC: MD-Anderson Cancer Center; BCM: Baylor College of Medicine; SG: Shanghaj GeneChem Co., Ltd.; BDB: Beijing Doing Biomedical Co. Ltd.; RH: RenJi Hospital; FHCRC: Fred Hutchinson Cancer Research Center; CPGH: Chinese PLA General Hospital; TFPHY: The First People's Hospital of Yunnan; HHUTCM: The Second Affiliated Hospital of Henan University of Traditional Chinese Medicine; HSB: Hebei Senlang Biotechnology; UU: Uppsala University; CLL: Chronic Lymphocytic Leukemia; SLL: Small Lymphocytic Lymphoma; ALL: Acute Lymphoblastic Leukemia; NHL: Non-Hodgkin Lymphoma.

activators of T-cell responses might be Dendritic Cells (DCs) as Antigen-Presenting Cells (APC). However, the therapeutic application of DCs is to be investigated, it is already known that DC potency varies between patients and this hampers the usage of them as a reliable source for T-cell activation [34]. The Artificial Antigen-Presenting Cells (AAPCs) are used as another option of cell-based T

cell activation [35]. While the selection of GMP-grade HLA-matched AAPC lines still requires additional resources, irradiated K562-derived AAPCs have been used to stimulate activation of CAR T-cells [36]. To simplify the *ex vivo* T-cell activation procedure many biotech companies have generated off-the-shelf clinical grade beads-based T-cell activation. It has also been used antibody-coated magnetic beads technology. Dynabeads CD3/CD28 are uniform super-paramagnetic beads covalently bind to CD3 and CD28 antibodies [16]. Miltenyi MACS introduced into the market antibody-coated nanobeads. They are polymeric nanomatrix conjugated to CD3 or to CD28 mAbs. The advantage of TransAct CD3/28 beads is that they are biodegradable, therefore they do not require removal prior to formulation but the upstream T-cell purification prior to activation is needed [37]. And the last but not least, adequate activation and expansion of patient peripheral blood mononuclear cells with anti-CD3 monoclonal antibody OKT3 for the production of autologous and allogenic CD19-CART cells has been reported. T-cell activation in this case should be supported by the presence of IL-2 [11,38].

Gene transfer systems in CAR T-cell therapy

There are four major types of stable gene expression vectors used for clinical applications: γ-retroviral vectors, lentiviral vectors, the transposone/transposase system and messenger RNA (mRNA) transfer-mediated gene expression.

γ-retroviral vectors were the first vectors that provide stable CD19 CAR expression [39]. They are broadly used in clinical trials requiring gene transfer delivery [40] because they provide high gene expression by means of the availability of multiple stable packaging cell lines with wide tropism [41,42].

The next group of vectors that mediate high gene transfer efficiency and drive stable level of CAR expression are lentiviral vectors. They are widely used as they can transduce nondividing cells – except for cells in G0 phase – and display a safer genomic integration profile in the context of genetically modified hematopoietic stem cells [43-45].

A relatively new plasmid based expression system, the transposone/transposase system, has been used to introduce anti-CD19 CAR into T cells by electroporation. The advantages of this system are its simple manufacturing procedure, as opposed to both retroviral and lentiviral vectors. The other advantages are relatively low cost and straightforward release testing. Ongoing anti-CD19 CAR T-cell trial using the sleeping beauty transposone/transposase system shows low T-cell toxicity [7,35,46].

Messenger RNA (mRNA) transfer provides a cytoplasmic expression system that enables transient expression of the transgene. *In vitro* transcribed mRNA can be introduced into cells by electroporation or by endocytosis. RNA transfection allows the expression of the transgene for about one week, and has been used to deliver mRNA for TCR/CAR, chemokine receptors and cytokines [47,48].

Clinical CAR T-cell results

CAR-mediated T cell responses may be further enhanced with addition of co-stimulatory domains. Preclinical studies showed that inclusion of potent signaling molecules improves the antitumor activity of genetically modified T cells [49]. It was found that inclusion of the CD137 (4-1BB) signaling domain significantly increased antitumor activity and *in vivo* persistence of CARs compared to inclusion of the CD3ζ chain alone [33,50]. To evaluate

the safety and feasibility for adoptive transfer of T cells gene-modified to express such CARs, Porter et al. [15] initiated a pilot clinical trial using autologous T cells expressing an anti-CD19 CAR including both CD3ζ and the 4-1BB co-stimulatory domain (CART19 cells) to target CD19+ malignancies. There were three patients (pts) enrolled into the study. Some of the findings from one of these pts' reports that the treatment results in tumor regression, CART19 cell persistence, and the unexpected occurrence of delayed tumor lysis syndrome. It was shown that the CART19 cells mediated potent clinical antitumor effects in all three pts treated [15]. On average, each infused CAR T-cell eliminated more than 1000 leukemia cells *in vivo* in pts with advanced chemotherapy-resistant CLL. CART19 cells underwent robust *in vivo* T cell expansion, persisted at high levels for at least 6 months in blood and Bone Marrow (BM), continued to express functional receptors on cells with a memory phenotype, and maintained anti-CD19 effector function *in vivo* [15].

Currently, most clinical trials reported to have used the second generations CARs with CD28 or/and CD137 co-stimulation [10,11,16,51] (Table 1). Third and fourth generation CARTs are in development and contain more than one co-stimulatory molecule with or without a suicide switch. Although most of these CARTs are still in the preclinical stage, at least one group has begun to evaluate fourth-generation [52]. The optimal CAR structure remains an area of active investigation, and it is possible that different targets of diseases would be best treated with different CAR constructs. The original studies done with 14 pts with relapsed and refractory CLL at the University of Pennsylvania [53] showed that majority of the pts were heavily treated with the range of 1 to 11 prior therapies and 43% of total group were 17p deleted. Autologous T cells transduced with a CD19-directed CAR (CTL019) lentiviral vector were infused into pts with relapsed/refractory CLL at doses of 0.14×10^8 to 11×10^8 CTL019 cells (median, 1.6×10^8 cells). Patients were monitored for toxicity, response, expansion, and persistence of circulating CTL019 T cells. What it really should be emphasised here is one important thing to understand that we need lymphocyte depleting therapy to really enable the CAR T-cells within go into actually have a sort of vacancy in the area to grow. And it is sort of interesting because B-cell disease is like a flair that can happen after B-cell depleting therapy with rituximab. The essence to capitalize on that right is actually bendamustin, which is not really meant to be back to the clone of CLL cells but really just creates the space so there is a push for more lymphocytes genesis. In the results obtained by Porter et al. [54] the overall response rate in these heavily pretreated CLL pts was 8 of 14 (57%), with 4 complete remissions (CR) and 4 partial remissions (PR). The *in vivo* expansion of the CAR T cells correlated with clinical responses, and the CAR T cells persisted and remained functional beyond 4 years in the first two pts achieving CR. No patient in CR has relapsed. All responding pts developed B cell aplasia and experienced cytokine release syndrome, coincident with T cell proliferation. Minimal residual disease was not detectable in pts who achieved CR, suggesting that disease eradication may be possible in some pts with advanced CLL [53]. But it really does not seem to be any relationship between the numbers of T cells infused to the overall response. One thing that it really does seem to actually predict for response is really persistence of the T cells. The data we have so far is getting a persistence of T-cells and is really going to be a key to get unsustainable engraftment and continue worst therapeutic benefit. Toxicities have been substantial, but there is a lot of trick how to control them [54]. No really infusion toxicities have been observed,

they really looks like a blood transfusion but most pts experience delayed Cytokine-Release Syndrome (CRS) characterized by high fevers, nausea, myalgia, capillary leak syndrome, hypoxemia and hypotension [54]. Unfortunately, this syndrome limits the widespread clinical use of this novel immunotherapy. CRS is characterized by not only high fevers but also a systemic inflammatory response that is at times fatal. CRS is associated with elevation of multiple inflammatory cytokines (IFNγ, TNFα, IL-6 and others) and may be treated with the anti-IL-6 receptor antibody tocilizumab and/or steroids. Importantly, approaches to prevent CRS are currently lacking. There is also available siltuximab, which is approved, but does seem to be used to actually help treat CRS without interfering with therapeutic benefit. Of course, steroids were also used very rarely because of obviously the urgency of control the situation but they were not shown to have some impact the actual therapeutic benefit of CAR T-cells. There are not many people in the study far out, but at U Penn they have some potential pts that can be put in the long term remissions with CART therapy. Patients who had CR had no MRD (Minimal Residual Disease) detected, so if they respond they respond extremely well. That is an important observation because the question of course is if there were people with residual lymph andenopathy is an active disease or not. Here we know that MRD negativity is actually a very well predictor of outcome. To paraphrase it, this is a very good indicator of a depth remission. Responding pts had persistence of CAR T-cells more than several months post-Rx. Patients with persistence of CAR T-cells had persistent absence of normal B cells and profound hypogammaglobulinemia (IgM and IgA levels of 0). Those pts required maintenance therapy with IV Ig [54]. Ruella et al. [55] has recently demonstrated that the rational combination of ibrutinib with CART19 leads to enhance anti-tumor responses in preclinical model of MCL, CLL and B-cell Acute Lymphoblastic Leukemia (ALL). In addition ibrutinib has been shown to modulate T cell cytokine production. They showed that ibrutinib reduces CART19-mediated CRS and prolongs survival by inhibiting the production of inflammatory cytokines from both CART and tumor cells. Having previously shown that ibrutinib does not impair T cell expansion *in vivo* and indeed enhances the anti-tumor effect, we suggest that the CART19-ibrutinib combination could be a novel strategy to prevent CRS in B-cell lymphomas as well as in B-cell acute lymphoblastic leukemia. U Penn recently opened clinical trial (NCT02640209) where CART19 (CTL019) is added to ibrutinib in CLL patients who have not achieved a complete response after 6 months [55]. Turtle et al. [56] reported similar outcomes. They treated 18 adults with CLL who had previously received ibrutinib with anti-CD19 CAR T-cells that were manufactured from defined CD4$^+$ and CD8$^+$ T cell subsets obtained by immunomagnetic selection of leukapheresis products, formulated in a final 1:1 ratio of CD8$^+$:CD4$^+$ CAR$^+$ T cells, and infused at 1 of 3 dose levels (2×10^5, 2×10^6 or 2×10^7 CAR-T cells/kg) after lymphodepletion chemotherapy (cyclophosphamide and fludarabine). All pts were refractory to or had relapsed after receiving a regimen containing fludarabine and rituximab, and all pts had previously received ibrutinib; 11 were ibrutinib-refractory, 3 were ibrutinib-intolerant, and 4 were refractory to venetoclax. Twelve pts had a complex karyotype and 11 pts had 17p deletion. The median percentage of abnormal B cells in marrow was 77% (range 0.4 Ð 96). All pts had extramedullary disease and 2 had CNS disease. Seventeen pts have completed response and toxicity assessment. The ORR was 76% (8 PR and 5 CR). Two of the pts with PR by lymph node size criteria (IWCLL 2008) had negative PET scans after therapy. Among ibrutinib-refractory (n=10) or intolerant pts (n=3), the ORR was

77% (7 PR and 3 CR). In venetoclax refractory pts, 2 of 4 responded (PR). The obtained results are very promising: high response rates and durable CRs in poor prognosis pts who have previously failed ibrutinib. They also reported that the use of CAR T-cells products with a prescribed 1:1 CD4/CD8 composition identified CAR T-cells doses associated with a reduced incidence and severity of these complications without impairing efficacy [57]. Geyer et al. [58] suggest that prior therapy with ibrutinib may influence end of process (EOP) CAR T-cells phenotypes. In total, 5 of 11 enrolled pts with CLL (45%) treated with CCT and 19-28z CAR T cells achieved objective response (Minimal Residual Disease [MRD]-CR, n=2; maintenance of MRD+CR, n=1; PR, n=2); ORR was 4/5 among IBR-treated pts (1 MRD-CR, 1 MRD+ CR, 2 PR; p=0.08 for ORR between IBR-treated vs IBR-naïve pts). Two pts remain in MRD- CR at 16 and 50 months. Maximal CAR T cell persistence observed to date was 159 days [58].

CARTs expanded several hundred folds *in vivo*, trafficked to tumor sites, produced cytokines, and resulted in elimination of bulky tumor masses. Each CART cell and its progeny were capable of killing thousands of tumor cells, and CART cells differentiated into memory T cells that persisted up to 4 years in some pts (the long-term B-cell aplasia in those patients may suggest that these persisting cells remain functional) [15]. Although, the therapy is not devoid of side effects, CART19 is a useful and promising approach for pts with relapsed/refractory CLL, and further development and optimization of this therapy for pts with CLL is warranted.

Toxicity of CAR T-Cell Therapy

The most frequent and most severe is B-cell aplasia, expected on-target off-tumor toxicity. Several unique toxicities occurring after CAR T-cell therapy, including CRS, Macrophage Activation Syndrome (MAS), and neurotoxicity [10,11,50,52], have emerged and continue to present diagnostic and management challenges [59].

ROR1 Receptor

There is a very interesting observation that should be emphasized. Some patients vaccinated with autologous CLL cells made anti-ROR1 autoantibodies. ROR1 (Receptor Tyrosine Kinase-like Orphan Receptor 1) is expressed on sub-populations of B-cell malignancies and solid tumors, but not by healthy B cells or normal post-partum tissues. Thus, adoptive transfer of T cells specific for ROR1 has potential to eliminate tumor cells and spare healthy tissues. To test this hypothesis, scientists from US San Diego developed CARs targeting ROR1 in order to generate T cells specific for malignant cells. Two Sleeping Beauty transposons were constructed with 2nd generation ROR1-specific CARs signaling through CD3ζ and either CD28 (designated ROR1RCD28) or CD137 (designated ROR1RCD137) and were introduced into T cells [59].

It provides a growth/survival advantage for CLL cells *in vitro* and *in vivo*. Some, but not all, patients with anti-ROR1 autoantibodies made anti-ROR1 with functional activity and they still do allow the clone to exist [60-62]. ROR1 evolutionary conserved to membrane protein type-I that expressed primarily during embryogenesis. It is not expressed on normal post-partum tissues [63,64]. ROR proteins are type I transmembrane receptor tyrosine kinases (Figure 2). Like other RTKs, they are predominantly located in the plasma membrane. The extracellular region of vertebrate ROR proteins contains an Immunoglobulin (Ig) domain, a Cysteine-Rich Domain (CRD), also called a Frizzled domain, and a Kringle (Kr) domain. Intracellularly, ROR proteins possess a Tyrosine Kinase (TK) domain, and a proline-
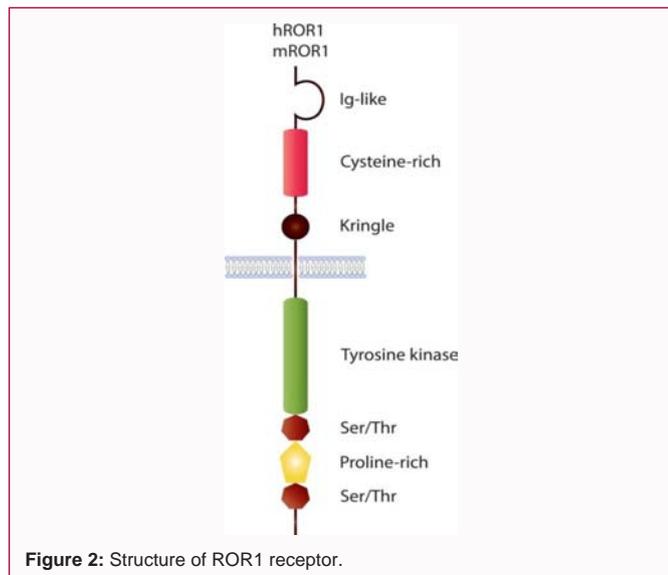
Figure 2: Structure of ROR1 receptor.

rich domain straddled by two serine-threonine-rich (S/T1 and S/T2) domains [65,66]. The extracellular CRD of ROR is similar to the Wnt-binding domain found in Frizzled receptors [67-69], suggesting that ROR proteins also bind to Wnt ligands. Although, several studies report diverse, and sometimes conflicting, interactions of ROR with Wnt signaling, Fukuda et al. [61] reported that ROR1 could bind Wnt5a, which induced activation of NF-kappaB when co-expressed with ROR1 in HEK293 cells and enhanced the survival of CLL cells *in vitro*, an effect that could be neutralized by post-treatment anti-ROR1 antisera. It has been concluded that patients with CLL can break immune tolerance to ROR1, which is an oncofetal surface antigen and survival-signaling receptor in this neoplastic disease [60].

However, ROR1 is expressed on hematogones, there found a rare subpopulation of precursor B cells, CD45^{-dm}CD10$^+$CD19$^+$ blasts similar to pre-B ALL. This cells population is ~ ≤ 0.25% of normal marrow, might be increased in pediatric marrow S/P myeloablative Rx [60,61]. The expression of ROR1 is very nicely restricted to CLL B-cells only. So the essence, that should be really improved the targeting of the CAR T-cells therapy is really trying diminish other toxicities. Berger et al. [70] reported that ROR1 CAR T-cells did not cause overt toxicity to normal organs and accumulated in bone marrow and lymph nodes sites where ROR1 positive B cells were presented. A major advantage of targeting ROR1 over the current T-cell therapies targeting CD19 is that recipients would not deplete B cells and develop hypogammaglobulinemia, thereby mitigating the risk for impaired humoral immunity [71,72]. T cells that express CARs specific for CD19 cannot distinguish between neoplastic and normal CD19-bearing B cells. Indeed, the initial clinical data has demonstrated that most patients benefiting from an anti-tumor response have concomitant B-cell depletion. Such B-cell aplasia requires that the patient receive timely intravenous infusions of normal immunoglobulin to alleviate the threat for opportunistic infections [73]. Nevertheless, such treatment cannot eliminate the risk for serious infection, as one recipient of CD19-specific CAR$^+$ T cells died due to opportunistic infection [74]. Thus, targeting a Tumor-Associated Antigen (TAA), which is not expressed on normal B cells or other adult tissues would mitigate the risk for B-cell depletion and potentially improve outcome. One TAA that may serve as an alternative to CD19-directed T-cell therapy is ROR1 [59]. The

efficacy and specificity of these CARs to CLL is very high. This specific targeted mode of therapy seems to be underway in its practical uses.

Summary

CART is a best in class immunology cancer treatment turning the immune system into a smart drug to seek, identify and destroy cancers. CAR T-cells can persist and expand in the body where the cells act like "living drug" - combining the specificity of antibodies with the killing power of T-cells. Immunotherapy with CD19 CAR T-cells of defined subset composition is feasible in patients with CLL and has potent anti-tumor activity. Toxicity is related to cell dose. Efficacy seems dependent on the ability of the enhanced cells to expand and persist in the body after infusion into the patients. The long-lasting clinical remissions and B-cell aplasia demonstrate the functional persistence of CD19 CARTs and highlight the potential for a paradigm shift in adoptive immunotherapy. A major advantage of targeting ROR1 (a tumor-associated antigen) over the current T-cell therapies targeting CD19 is that patients would not deplete B cells and develop hypogammaglobulinemia, thereby mitigating the risk for impaired humoral immunity. This relatively new therapy is very promising but also has many challenges. Adoptive CAR T-cell therapy will hopefully prove to be as effective in solid tumors as in onco-hematological indications. The efficacy of new tumor targets for CAR T-cells expanded from CD19 to a great range of new targets including CD20, CD22, CD30, CD33, CD138, CD171, CEA, EGFRvIII, ErbB, FAP, GD2, HER2, glypican 3, mesothelin, and NKG2D [7,23]. The greatest challenge is also provide new sources of T cells to obtain autologous T cells [75]. The manufacturing with defined subpopulations of T cells that can be derived from a blood draw instead of leukapheresic product would reduce the scale and therefore the cost of manufacturing.

Acknowledgments

References

1. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med. 2005;352(8):804-15.

2. Nagasawa T. Microenvironmental niches in the bone marrow required for B-cell development. Nat Rev Immunol. 2006;6(2):107-16.

3. http://www.whatisbiotechnology.org/exhibitions/milstein

4. Plosker GL, Figgitt DP. Rituximab: a review of its use in non-Hodgkin's lymphoma and chronic lymphocytic leukaemia. Drugs. 2003;63(8):803-43.

5. Irving BA, Weiss A. The cytoplasmic domain of the T cell receptor zeta chain is sufficient to couple to receptor-associated signal transduction pathways. Cell. 1991;64(5):891-901.

6. Sadelain M, Brentjens R, Rivière I. The promise and potential pitfalls of chimeric antigen receptors. Curr Opin Immunol. 2009;21(2):215-23.

7. Wang X, Riviere I. Clinical manufacturing of CAR T cells: foundation of a promising therapy. Mol Ther Oncolytics. 2016;3:16015.

8. Brentjens RJ, Davila ML, Riviere I, Park J, Wang X, Cowell LG, et al. CD-19targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia. Sci Transl Med. 2013;5(177):177ra38.

9. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia.

N Engl J Med. 2013;368:1509-18.

10. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. Sci Trans Med. 2014;6(224):224ra25.

11. Kochenderfer JN, Dudley ME, Kassim SH, Somerville RP, Carpenter RO, Stetler-Stevenson M, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. J Clin Oncol. 2015;33(6):540-9.

12. Grupp SA, Mande SL, Shaw PA, Aplenc R, Barret D, Callahan C, et al. Durable remissions in children with relapsed/refractory ALL treated with T cells engineered with a CD19-targeted chimeric antigen receptor (CTL019). Blood. 2015;126:681.

13. Topp MS, Gökbuget N, Zugmaier G, Klappers P, Stelljes M, Neumann S, et al. Phase II trial of the anti-CD19 bispecific T cell-engager blinatumomab shows hematologic and molecular remissions in patients with relapsed or refractory B-precursor acute lymphoblastic leukemia. J Clin Oncol. 2014;32(36):4134-40.

14. Uckun FM, Jaszcz W, Ambrus JL, Fauci AS, Gajl-Peczalska K, Song CW, et al. Detailed studies on expression and function of CD19 surface determinant by using B43 monoclonal antibody and the clinical potential of anti-CD19 immunotoxins. Blood. 1988;71(1):13-29.

15. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. N Engl J Med. 2011;365(8):725-33.

16. Lee DW, Kochenderfer JN, Stetler-Stevenson M, Cui YK, Dellbrook C, Feldman SA, et al. T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose escalation trial. Lancet. 2015;385(9967):517-28.

17. Flemming A. Deal watch: Pfizer and GSK join race for T cell cancer therapies. Nat Rev Drug Discov. 2014;13(8):568-9.

18. June CH, Riddell SR, Schumacher TN. Adoptive cellular therapy: a race to the finish line. Sci Transl Med. 2015;7(280):280ps7.

19. Gill S, Tasian SK, Ruella M, Shestova O, Li Y, Porter DL, et al. Preclinical targeting of human acute myeloid leukemia and myeloablation using chimeric antigen receptor-modified T cells. Blood. 2014;123(15):2343-54.

20. Savoldo B, Rooney CM, Di Stasi A, Abken H, Hombach A, Foster AE, et al. Epstein Baar virus specific cytotoxic T lymphocytes expressing the anti-CD30 zeta artificial chimeric T-cell receptor for immunotherapy of Hodgkin disease. Blood. 2007;110(7):2620-30.

21. Kochenderfer JN, Feldman SA, Zhao Y, Xu H, Black MA, Morgen RA, et al. Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor. J Immunother. 2009;32(7):689-702.

22. Anwer F, Shaukat AA, Zahid U, Husnain M, McBride A, Persky D, et al. Donor origin CAR T cells: graft versus malignancy effect without GVHD, a systemic review. Immunotherapy. 2017;9(2):123-30.

23. Gill S, Maus MV, Porter DL. Chimeric antigen receptor T cell therapy: 25years in the making. Blood Rev. 2016;30(3):157-67.

24. Ahmed N, Brawley VS, Hegde M, Robertson C, Ghazi A, Gerken C, et al. Human Epidermal Growth Factor Receptor 2 (HER2) - specific chimeric antigen receptor - modified T-cells for the immunotherapy of HER2 - positive sarcoma. J Clin Oncol. 2015;33(15):1688-96.

25. Koneru M, O'Cearbhaill R, Pendharkar S, Spriggs DR, Brentjens RJ. A phase I clinical trial of adoptive T cell therapy using IL-12 secreting MUC-16 (ecto) directed chimeric antigen receptors for recurrent ovarian cancer. J Transl Med. 2015;13:102.

26. Hassan R, Bullock S, Premkumar A, Kreitman RJ, Kindler H, Willingham MC, et al. Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bollus IV infusion to patients with mesothelin expressing mesothelioma, ovarian and pancreatic cancer. Clin Cancer Res. 2007;13(17):5144-9.

27. Wang X, Naranjo A, Brown CE, Bautista C, Wong CW, Chang WC, et al. Phenotypic and functional attributes of lentivirus-modified CD19-specific human CD8+ central memory T cells manufactured at clinical scale. J Immunother. 2012;35(9):689-701.

28. Casati A, Varghaei-Nahvi A, Feldman SA, Assenmacher M, Rosenberg SA, Dudley ME, et al. Clinical-scale selection and viral transduction of human naïve and central memory CD8+ T cells for adoptive cell therapy of cancer patients. Cancer Immunol Immunother. 2013;62(10):1563-73.

29. Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S, et al. Multiplex genome-edited T cell manufacturing platform for "Off-the-Shelf" adoptive t-cell immunotherapies. Cancer res. 2015;75(18):3853-64.

30. Guedan S, Chen X, Madar A, Carpenito C, McGettigan SE, Frigault MJ, et al. ICOS-based chimeric antigen receptors program bipolar TH17/TH1 cells. Blood. 2014;124(7):1070-80.

31. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer Discov. 2013;3(4):388-98.

32. Hombach AA, Heiders J, Foppe M, Chmielewski M, Abken H. OX40 costimulation by a chimeric antigen receptor abrogates CD28 and IL-2 induced IL-10 secretion by redirected CD4(+) T cells. Oncoimmunology. 2012;1(4):458-466.

33. Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther. 2009;17(8):1453-64.

34. Vacchelli E, Vitale I, Eggermont A, Fridman WH, Fuciková J, Cremer I, et al. Trial watch: Dendritic cell-based interventions for cancer therapy. Oncoimmunology. 2013;2(10):e25771.

35. Kim JV, Latouche JB, Rivière I, Sadelain M. The ABCs of artificial antigen presentation. Nat Biotechnol. 2004;22(4):403-10.

36. Singh H, Huls H, Kebriaei P, Cooper LJ. A new approach to gene therapy using Sleeping Beauty to genetically clinical-grade T cells to target CD19. Immunol Rev. 2014;257(1):181-90.

37. Wang X, Stefanski J, Borquez-Ojeda O, Qu J, Hack A, He Q, et al. Comparison of CTS Dynabeads CD3/CD28, Miltenyi TransAct CD3/28 and ExpAct beads for large-scale CART cell manufacturing. European Society of Gene and Cell Therapy Collaborative Congress 2015; Helsinki, Finland. A31.

38. Brudno JN, Somerville RP, Shi V, Rose JJ, Halverson DC, Fowler DH, et al. Allogenic T-cells that express an anti-CD19 chimeric antigen receptor induce remission of B-cell malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. J Clin Oncol. 2016;34(10):1112-21.

39. Brentjens RJ, Latouche JB, Santos E, Marti F, Gong MC, Lyddane C, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med. 2003;9(3):279-86.

40. Deichmann A, Schmidt M. Biosafety considerations using gamma-retroviral vectors in gene therapy. Curr Gene Ther. 2013;13(6):469-77.

41. Ghani K, Wang X, de Campos-Lima PO, Olszewska M, Kamen A, Riviere I, et al. Efficient human hematopoietic cell transduction using RD114- and GALV-pseudotyped retroviral vectors produced in suspension and serum-free media. Hum Gene Ther. 2009;20(9):966-74.

42. Miller AD, Garcia JV, von Suhr N, Lynch CM, Wilson C, Eiden MV. Construction and properties of retrovirus packaging cells based on gibbon ape leukemia virus. J Virol. 1991;65(5):2220-4.

43. Naldini L, Blömer U, Gallay P, Ory D, Mulligan R, Gage FH, et al. In vivo gene delivery and stable transduction of nondividing cells by a lentiviral vector. Science. 1996;272(5259):263-7.

44. Vannucci L, Lai M, Chiuppesi F, Ceccherini-Nelli L, Pistello M. Viral vectors: a look back and ahead on gene transfer technology. New Microbiol. 2013;36(1):1-22.

45. Merten OW, Hebben M, Bovolenta C. Production of lentiviral vectors. Mol Ther Methods Clin Dev. 2016;3:16017.

46. Vargas JE, Chicaybam L, Tetelbom Stein R, Tanuri A, Delgado-Cañedo A, Bonamino MH. Retroviral vectors and transposons for stable gene therapy: advances, current challenges and perspectives. J Translation Med. 2016;14(1):288.

47. Zhao Y, Zheng Z, Cohen CJ, Gattinoni L, Palmer DC, Restifo NP, et al. High-efficiency transfection of primary human and mouse T lymphocytes using RNA electroporation. Mol Ther. 2006;13(1):151-9.

48. Yoon SH, Lee JM, Cho HI, Kim EK, Kim HS, Park MY, et al. Adoptive immunotherapy using human peripheral blood lymphocytes transferred with RNA encoding Her-2/neu-specific chimeric immune receptor in ovarian cancer xenograft model. Cancer Gene Ther. 2009;16(6):489-97.

49. Dotti G, Gottschalk S, Savoldo B, Brenner MK. Design and development of therapies using chimeric antigen receptor - expressing T cells. Immunol Rev. 2014;257(1):107-26.

50. Carpenito C, Milone MC, Hassan R, Simonet JC, Lakhal M, Suhoski MM, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. Proc Natl Acad Sci U S A. 2009;106(9):3360-5.

51. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371:1507-17.

52. Dong L, Chang L-J, Gao Z, Lu DP, Zhang JP, Wang LP, et al. Chimeric antigen receptor 4SCAR19-modified T cells in acute lymphoid leukemia: a phase II multi-center clinical trial in China. Blood. 2015;126:3774.

53. Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med. 2015;7(303):303ra139.

54. Porter DL et al. Blood 122:873 ASH Proceedings 2013

55. Ruella M, Kenderian SS, Shestova O, Klichinsky M, Melenhorst JJ, Wasik MA, et al. Kinase Inhibitor Ibrutinib Prevents Cytokine-Release Syndrome after Anti-CD19 Chimeric Antigen Receptor T Cells (CART) for B Cell Neoplasms. ASH poster, Blood. 2016;128:2159.

56. Turtle JC, Hanafi L-A, Li D, Chaney C, Heimfeld S, Riddell SR, Maloney DG. CD19 CAR-T Cells Are Highly Effective in Ibrutinib-Refractory Chronic Lymphocytic Leukemia. ASH Abstract, Blood. 2016;128:56.

57. Turtle CJ, Hay KA, Juliane G, Hanafi L-A, Li D, Chaney C, et al. Biomarkers of Cytokine Release Syndrome and Neurotoxicity after CD19 CAR-T Cells and Mitigation of Toxicity By Cell Dose. ASH Abstract, Blood. 2016;128:1852.

58. Geyer MB, Park JH, Riviere I, Senechal B, Wang X, Purdon TJ, et al. Implications of Concurrent Ibrutinib Therapy on CAR T-Cell Manufacturing and Phenotype and on Clinical Outcomes Following CD19-Targeted CAR T-Cell Administration in Adults with Relapsed/Refractory CLL. ASH Abstract, Blood. 2016;128:58.

59. Kenderian SS, Porter DL, Gill S. Chimeric Antigen Receptor T Cells and Hematopoietic Cell Transplantation: How Not to Put the CART Before the Horse. Biol Blood Marrow Transplant. 2017;23(2):235-46.

60. Deniger DC, Yu J, Huls MH, Figliola MJ, Mi T, Maiti SN, et al. Sleeping Beauty Transposition of Chimeric Antigen Receptors Targeting Receptor Tyrosine Kinase-Like Orphan Receptor-1 (ROR1) into Diverse Memory T-Cell Populations. PLoS One. 2015;10(6):e0128151.

61. Fukuda T, Chen L, Endo T, Tang L, Lu D, Castro JE, et al. Antisera induced by infusions of autologous Ad-CD154-leukemia B cells identify ROR1 as an oncofetal antigen and receptor for Wnt5a. Proc Natl Acad Sci U S A. 2008;105(8):3047-52.

62. Broome HE, Rassenti LZ, Wang HY, Meyer LM, Kipps TJ. ROR1 is expressed on hematogones (non-neoplastic human B-lymphocyte precursors) and a minority of precursor-B acute lymphoblastic leukemia. Leuk Res. 2011;35(10):1390-4.

63. Zhang S, Cui B, Lai H, Liu G, Ghia EM, Widhopf GF, et al. Ovarian cancer stem cells express ROR1, which can be targeted for anti-cancer-stem-cell therapy. Proc Natl Acad Sci U S A. 2014;111(48):17266-71.

64. Green JL, Kuntz SG, Sternberg PW. Ror receptor tyrosine kinases: orphans no more. Trends Cell Biol. 2008;18(11):536-44.

65. Matsuda T, Nomi M, Ikeya M, Kani S, Oishi I, Terashima T, et al. Expression of the receptor tyrosine kinase genes, Ror1 and Ror2, during mouse development. Mech Dev. 2001;105(1-2):153-6.

66. Masiakowski P, Carroll RD. A novel family of cell surface receptors with tyrosine kinase-like domain. J Biol Chem. 1992;267(36):26181-90.

67. Saldanha J, Singh J, Mahadevan D. Identification of a Frizzled-like cysteine rich domain in the extracellular region of developmental receptor tyrosine kinases. Protein Sci. 1998;7(8):1632-5.

68. Xu YK, Nusse R. The Frizzled CRD domain is conserved in diverse proteins including several receptor tyrosine kinases. Curr Biol. 1998;8(12):R405-6.

69. Masiakowski P, Yancopoulos GD. The Wnt receptor CRD domain is also found in MuSK and related orphan receptor tyrosine kinases. Curr Biol. 1998;8(12):R407.

70. Berger C, Sommermeyer D, Hudecek M, Berger M, Balakrishnan A, Paszkiewicz PJ, et al. Safety of targeting ROR1 in primates with chimeric antigen receptor-modified T cells. Cancer Immunol Res. 2015;3(2):206-16.

71. Singh H, Figliola MJ, Dawson MJ, Huls H, Olivares S, Switzer K, et al. Reprogramming CD19-specific T cells with IL-21 signaling can improve adoptive immunotherapy of B-lineage malignancies. Cancer Res. 2011;71(10):3516-27.

72. Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. Blood. 2010;116(20):4099-102.

73. Jolles S, Sewell WA, Misbah SA. Clinical uses of intravenous immunoglobulin. Clin Exp Immunol. 2005;142(1):1-11.

74. Brentjens R, Yeh R, Bernal Y, Riviere I, Sadelain M. Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. Mol Ther.