



# Chimeric T-cell receptors: new challenges for targeted immunotherapy in hematologic malignancies

Ettore Biagi, Virna Marin, Greta Maria Paola Giordano Attianese, Erica Dander, Giovanna D'Amico, Andrea Biondi

## ABSTRACT

"Centro di Ricerca Matilde Tettamanti", Department of Paediatrics, University of Milano-Bicocca, San Gerardo Hospital, Monza, Milan, Italy.

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Correspondence:  
Ettore Biagi, Centro di Ricerca "M. Tettamanti", Clinica Pediatrica Università Milano-Bicocca, Via Pergolesi 33, 20052 Monza.  
E-mail: [ettore.biagi@pediatrionza.it](mailto:ettore.biagi@pediatrionza.it)

Chimeric T-cell receptors (ChTCR) are a fascinating technological step in the field of immunotherapy for orienting the activity of immune cells towards specific molecular targets expressed on the cell surface of various tumors, including hematologic malignancies. The main characteristics of ChTCR are their ability to redirect T-cell specificity and their killing/effector activity toward a selected target in a non-MHC-restricted manner, exploiting the antigen binding properties of monoclonal antibodies. ChTCR are, in fact, artificial T-cell receptors constituted by an antigen-recognizing antibody molecule linked to a T-cell triggering domain. Various hematologic malignancies represent optimal targets for the exploitation of ChTCR, because of the bright expression of specific antigens on the surface of tumor cells. Thus, CD19 and CD20 have been targeted for B-cell lymphoid tumors (acute lymphoblastic leukemia-ALL, lymphomas and chronic lymphocytic leukemia-CLL), CD33 for myeloid leukemia, and CD30 for lymphomas. Even though technical and safety progresses are still needed to improve the profile of gene transfer and protein expression of ChTCR, phase 1 trials will be carried out in the near future to demonstrate the feasibility of their clinical translation and, it is hoped, give preliminary indications about their anti-tumor efficacy.

Key words: chimeric T-cell receptor, immunotherapy, hematologic malignancy.

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Even though tumor immunotherapy is one of the most attractive and fascinating fields of modern medicine, clinical applications in humans have shown a limited number of significant responses and revealed many scientific and practical difficulties relating to the translation of apparently flawless *in vitro* or animal models to the setting of human cancers.<sup>1-4</sup>

Comprehension of the mechanisms of tumor escape represents one of the most relevant acquisitions in immunology and has opened a new challenge for scientists and physicians.<sup>5,6</sup> Tumor cells, in fact, evade recognition and elimination by immune effectors in many ways: i) low or absent expression of tumor-specific antigens; ii) expression of antigens that are shared with normal cells at certain developmental stages, so that the immune sys-

tem has become self-tolerant or anergic; iii) down-regulation of surface expression of MHC molecules; iv) defective pathways of antigen processing and presentation; v) absence of appropriate co-stimulation to deliver a complete activation stimulus to effector T cells; vi) the presence of inhibitory molecules actively secreted by the tumor itself or by the tumor microenvironment (such as interleukin-10 and transforming growth factor- $\beta$ );<sup>7</sup> and vii) the expansion of naturally occurring or tumor-induced T cells with regulatory activity.<sup>8,9</sup>

Besides being capable of efficiently overcoming these multiple mechanisms of immune escape, any application of immunotherapy must consider all the practical issues related to the production of immune cells for clinical use: i) the choice of the right antigen(s) to ensure use in a

sufficiently wide category of patients; ii) the development of methods of manipulation, which should be consistently straightforward and not excessively time-consuming, in order to obtain large numbers of cells in a suitable framework; iii) the use of appropriate Good Manufacturing Practice (GMP)-grade reagents and procedures which guarantee the exploitation of rigid rules of cell manipulation;<sup>10</sup> iv) the consideration of safety issues related to the possible occurrence of genetic aberrations when using vectors for gene delivery.<sup>11</sup>

Chimeric T-cell receptors (ChTCR) represent a valuable tool for overcoming the above mentioned obstacles and are certainly an attractive and promising line of medical research, whose clinical application in the near future may give new prospects to tumor immunotherapy.<sup>12-16</sup> The main characteristics of ChTCR are their ability to redirect T-cell specificity and their killing/effector activity toward a selected target in a non-MHC-restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. ChTCR are, in fact, artificial T-cell receptors constituted by an antigen-recognizing antibody molecule linked to a T-cell triggering domain.

The scope of this review is not to describe the technical details concerning the construction of these artificial molecules (reviewed elsewhere<sup>13</sup>), but to illustrate their functional immune aspects and their current or potential impacts on immunotherapy for hematologic malignancies.

### **Use of ChTCR-expressing T cells for tumor immunotherapy**

Adoption of virus-specific T cells (cytotoxic T-lymphocytes, CTL) has demonstrated that clinical applications of immunotherapy are feasible and effective when a strong antigen (or a pool of different antigens) is recognized by the immune effectors (the most successful attempts have involved Epstein-Barr virus [EBV] and cytomegalovirus [CMV]-specific CTL).<sup>17-20</sup> Unfortunately, in the context of tumors, the specific antigens are often weak, poorly expressed on the cell surface, or presented in an inappropriate or incomplete way, often accompanied by secretion of inhibitory T-cell factors. Moreover, most tumors can elude major histocompatibility complex (MHC)-restricted T-cell-mediated immune recognition.<sup>5,6</sup> For these reasons, enrichment and expansion of CTL targeting tumor-associated antigens are time-consuming and often ineffective, due to the low frequency of tumor-specific precursors *in vivo*.<sup>16</sup>

Gene manipulation by ChTCR offers the means, in a unique molecule, of augmenting the T-cell immune properties, so that T-cell reactivity can be artificially driven towards selected antigens and their survival, in a hostile tumor milieu, is strongly improved by the addition of endogenous growth factors and co-stimulation signals, or by blocking T-cell inhibitory or pro-apoptotic pathways.<sup>12-16</sup> Several artificial ChTCR have been devised in the last decade.<sup>21</sup> These molecules are con-

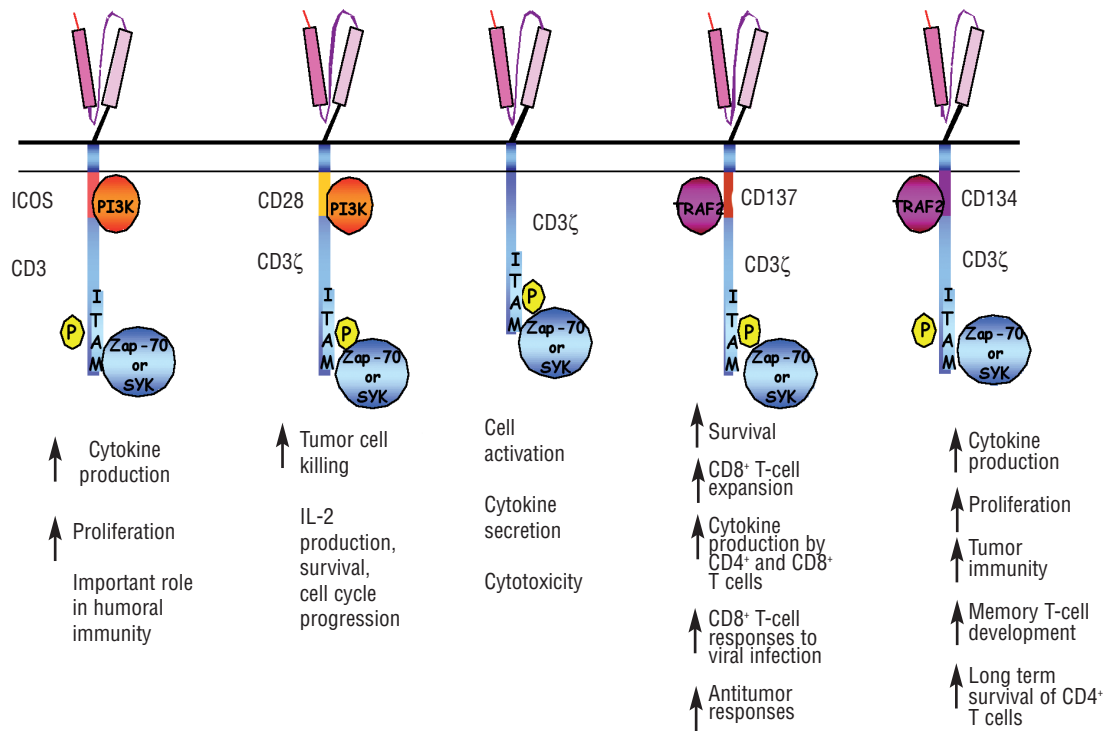
structed to express a specific antigen-binding domain (the extracellular domain, consisting of the variable chains of a monoclonal antibody), linked together as a single chain Fv (scFv), and a signaling region (the intracellular domain), usually taken from the zeta-chain of the TCR/CD3 complex (Figure 1). When expressed by T cells, the chimeric receptor links up the targeted antigen and triggers the cytolytic cascade of T cells, thus consecutively inducing the killing of the target population.<sup>22,23</sup> The main advantage of this approach is that it relies on the construction of a universal receptor towards a selected single molecule, whose recognition is non-MHC-restricted and independent of antigen processing, thus bypassing all major mechanisms of tumor escape.

### **Applications of ChTCR in the context of hematologic malignancies**

Various hematologic malignancies represent optimal targets for the exploitation of ChTCR, because of the expression of specific antigens on the surface of tumor cells, for which monoclonal antibodies are available.<sup>24,25</sup> Thus, CD19 and CD20 have been targeted for B-cell lymphoid tumors (acute lymphoblastic leukemia-ALL, lymphomas and chronic lymphocytic leukemia-CLL), CD33 for myeloid leukemia, and CD30 for lymphomas.

ChTCR targeting CD19 certainly represents the best example of application of this strategy for hematologic tumors and also gives an interesting historical perspective of the evolution of this technology.<sup>24,25</sup> The first requirement to redirect T cells towards a selected tumor target is the identification of an appropriate molecule, which is selectively expressed on cancer cells. With regard to lymphoid tumors of B-cell origin, CD19 is an ideal target, since it is present on virtually all leukemia cells in almost all cases.<sup>16,25</sup> Among hematopoietic cells, CD19 is expressed only on cells belonging to the B-cell compartment. For this reasons, several groups have investigated its use in both *in vitro* and *in vivo* animal models.

Jensen, Cooper and colleagues (City of Hope National Medical Center, Duarte, CA, USA) and Brentjens and colleagues (Memorial Sloan-Kettering Cancer Center, New York, NY, USA) were among the first to demonstrate the efficacy of CD19-positive target killing by redirected CD19-ChTCR-expressing T cells, corroborating the *in vitro* data with a NOD/SCID mice model.<sup>25-29</sup> The initial construct was composed of a CD19-specific single-chain immunoglobulin extracellular targeting domain, fused to a CD3-zeta intracellular signaling domain. It was shown that CD19-redirected CTL were capable of potently killing primary B-ALL blasts and also of producing Th1 cytokines and were consistently proliferating after recognition of the targeted molecule.<sup>26,27</sup> With the aim of translating this approach into a clinical strategy for relapsed B-ALL, Jensen's group recently focused on umbilical cord-blood transplanta-



**Figure 1.** Schematic representation of ChTCR structure with different signaling domains. The signaling domain of ChTCR may be modified to include co-stimulatory signals that improve the activation of ChTCR-expressing cells. Two major classes of co-stimulatory molecules can be distinguished: the B7 receptor family including CD28 and ICOS, recruiting PI3Kinase,<sup>63</sup> and the TNFR family, structurally different from the previous one, whose signaling is initiated through TRAF2 engagement.<sup>64</sup>

tion (UCBT), designing a method that complies with principles of current GMP for phase I clinical trials to ensure the identity, purity, potency and safety of the cellular product. UCBT-derived T cells were efficiently redirected towards the CD19<sup>+</sup> tumor target cells (including B-ALL) by electroporation with a plasmid encoding the CD19-ChTCR, also containing a suicide gene (the herpes virus 1 thymidine kinase, HSV-1 TK, gene).<sup>28</sup> Cells were efficiently expanded in relevant numbers for clinical intervention and afterwards cryopreserved for quality controls. CD19-ChTCR-expressing UCBT-derived T cells were capable of CD19-specific killing activity and cytokine secretion *in vitro*, as well as inducing regression of CD19<sup>+</sup> tumors in NOD/SCID mice, and being selectively eliminated *in vivo* after administration of gancyclovir.<sup>28</sup> This approach has recently been extended to autologous cell therapy for patients affected by follicular lymphoma, showing that *ex vivo* cell expansion from cryopreserved cell banks was sufficient to produce doses of between  $5 \times 10^9$  and  $1 \times 10^{10}$  engineered T cells for each cycle of production.<sup>29</sup> This manufacturing strategy is therefore suitable for producing gene-manipulated T cells for phase I clinical trials in the context of B-cell lymphoid malignancies.

Brenner's group at the Center of Cell and Gene Therapy (CAGT, Baylor College of Medicine, Houston,

TX, USA) has also widely investigated the immune properties and possible clinical applications of CD19-redirectioned T cells, developing methods of large scale clinical-grade production of retroviral vectors containing the CD19-ChTCR for stable integration into transduced T cells.<sup>12-16,30-32</sup> The most relevant findings obtained by this group are: i) the demonstration that the CD3- $\zeta$  signaling domain is not sufficient *per se* to guarantee complete and prolonged activation of manipulated T cells and that the integration of the signal transduction domain of the co-stimulatory molecule CD28 enhances the proliferative properties of the gene-modified T cells; ii) the fact that *dual-specific* T cells offer an improved chance of obtaining a larger *in vivo* expansion of engineered T cells and a long-lasting maintenance of a CD19-redirectioned T-cell memory pool. In particular, they used EBV-specific CTL. In fact, EBV infection is very common in humans and triggers the generation of high levels of EBV-specific CD4<sup>+</sup> T helper and CD8<sup>+</sup> cytotoxic T cells. *Dual-specific* T cells recognize EBV-infected cells through their conventional native T-cell receptor and the leukemia CD19<sup>+</sup> target through the artificial ChTCR. Using this stratagem, the engagement of the native TCR *in vivo* by recurrent EBV infections is capable of constantly stimulating the ChTCR – redirectioned T cells, assuring appropriate activation of all pathways of

T-cell stimulation, thereby also maintaining the lytic activity of the CD19-specific artificial T-cell receptor.

Instead of manipulating conventional T cells, Campana and colleagues (St. Jude Children's Research Hospital, Memphis, Tennessee, USA) have explored the use of natural killer (NK) cells.<sup>33,34</sup> NK may improve the therapeutic potential of allogeneic hematopoietic transplantation, but numerous published data have demonstrated that their efficacy is strongly diminished by the presence of various inhibitory HLA types. The authors therefore transduced the CD56<sup>+</sup>CD3<sup>-</sup> NK cells with a chimeric receptor directed against CD19. Relevant numbers of NK cells can be obtained in a relatively short time by culturing peripheral blood mononuclear cells with K562 cells expressing the NK-stimulatory molecules 4-1BB ligand and interleukin 15. Anti-CD19 ChTCR-expressing NK cells have a markedly enhanced capacity to kill B-ALL blasts. This model also corroborates the necessity to add a co-stimulatory signal to the CD3- $\zeta$  domain. Addition of 4-1BB is, in fact, followed by increased cell activation, with subsequent secretion of interleukin-2 and interferon- $\gamma$ .

Using the same retroviral vector kindly provided by Campana and colleagues, our group has recently investigated the efficacy of the CD19-ChTCR in the context of B-cell malignancies, transducing *ex vivo* expanded cytokine induced killer (CIK) cells, which are enriched in CD3<sup>+</sup>CD56<sup>+</sup> CD1d-unrestricted NK-T cells.<sup>35</sup> Such cells present peculiar characteristics that render them an attractive target for leukemia immunotherapy, since they have the intrinsic capability of reaching leukemia-infiltrated tissues, primarily the bone marrow. Our results show that CD19-ChTCR-expressing CIK cells not only become capable of efficiently killing otherwise resistant B-ALL cells, but also present high expression levels of adhesion molecules (CD49d and CD11a) and chemokine receptors (CXCR4, CCR6 and CCR7). They also show robust *in vitro* chemotactic activity towards their corresponding ligands, prominent adhesion and transmigration across endothelium and metalloproteinase-dependent invasion of basement membrane in response to CXCL12. All this reflects their potential capability to migrate into sites of B-ALL accumulation and therefore supports the hypothesis that CD19-ChTCR-redirectioned CIK cells are an interesting tool for B-ALL immunotherapy.<sup>35</sup> Moreover, from the stand point of application, CIK cells are attractive because of the reproducible and straightforward method for their generation and expansion, which only requires GMP-grade cytokines. Thus, large numbers of cells can be rapidly expanded in a closed system with minimal manipulation.

Like CD19, the CD20 antigen has also been chosen to target B-cell lymphoid malignancies by ChTCR.<sup>36,37</sup> The use of the monoclonal anti-CD20 antibody rituximab induces remissions in approximately 60% of patients

with relapsed follicular lymphoma; however, most patients eventually relapse despite continued expression of CD20 on lymphoma cells. A cellular immunotherapy strategy targeting CD20<sup>+</sup> cells by ChTRC may provide a more effective mechanism for eliminating lymphoma cells than anti-CD20 antibodies. As for the CD19-ChTCR, Jensen and colleagues electroporated peripheral blood mononuclear cells with a plasmid containing a CD20-specific scFvFc: $\zeta$  ChTCR. CD8<sup>+</sup> CTL clones were generated and showed specific killing capacity towards CD20<sup>+</sup> target cells, including primary tumor cells from patients affected by follicular lymphoma, small lymphocytic lymphoma, splenic marginal zone lymphoma, diffuse large B cell lymphoma, and CLL.<sup>37</sup> Cell numbers were adequate for clinical use. In view of these findings, a phase I clinical trial for relapsed follicular lymphoma is being initiated by Jensen's group.

As for B-origin malignancies, myeloid leukemias can also be targeted by a ChTCR towards the myeloid antigen CD33. Recently published *in vitro* data showed that human NK cells can be efficiently manipulated by electroporation, transferring a humanized chimeric immunoglobulin T-cell receptor to CD33. CD33-ChTCR-expressing NK cells specifically lysed the acute myeloid leukemia cell line KG1.<sup>38</sup>

Finally, an attractive molecule for targeting Hodgkin's lymphoma tumor cells is the CD30 antigen. In fact, Hodgkin and Reed-Sternberg cells express high amounts of the cell surface antigen CD30. Hombach and colleagues used a retroviral vector containing a ChTCR gene encoding for an extracellular domain consisting of the single-chain antibody fragment HRS3-scFv with specificity for the CD30 antigen. Specific binding of the CD30-ChTCR resulted in cytolytic MHC-unrestricted reactivity against CD30<sup>+</sup> tumor cells *in vitro*, thus offering an attractive model for adoptive cellular immunotherapy to be used in the context of resistant Hodgkin's disease.<sup>39,40</sup>

Even though many different molecules have been explored for human hematologic malignancies and clinical trials are ongoing or about to commence, unfortunately no data are yet available in the setting of human hematologic malignancies. However, ChTCR have been used in the setting of patients infected by immunodeficiency virus (HIV). In HIV patients, T cells have been transduced to express a ChTCR containing the extracellular domain of human CD4 which is capable of linking up the viral gp120 protein in order to recognize and eliminate HIV-infected cells. Although ChTCR-transduced T cells were functionally active *in vitro* and homed to the HIV-infected tissues, the anti-viral efficacy of manipulated cells was negligible.<sup>41,42</sup> In the field of tumor immunotherapy, the use of engineered T cells expressing artificial TCR specific for tumor-associated antigens has recently given promising and exciting results in the treatment of metastatic melanoma. Even though the

**Table 1.** A summary of the current major limitations of ChTCR technology and possible ways of overcoming them to improve ChTCR efficacy *in vivo*.

<i>ChTCR limitation</i>	<i>Proposed solution</i>
Short-life duration of injected ChTCR-expressing T cells due to the host's immune response to foreign proteins.	Construction of new-generation ChTCR by use of fully human recombinant single-chain antibodies in the extracellular binding domain.
Absence of T-helper CD4 <sup>+</sup> cells to sustain long-term cytotoxic activity.	Simultaneous administration of both CD4 <sup>+</sup> and CD8 <sup>+</sup> ChTCR-modified T cells.
Poor T-cell activation and proliferation after antigen-binding, due to limited signaling capacity.	Modification of the intracellular domain by introduction of <i>src family kinase lck</i> to promote the creation of a superior signal-transducing complex.
Lack of appropriate T-cell co-stimulation.	Addition of co-stimulatory molecules (CD28, ICOS, CD134, or CD137).
Inappropriate migration into sites of tumor infiltration.	Use of target cells with innate properties to migrate into sites of leukemia invasion (CIK cells).
Insertional mutagenesis after gene transfer by viral vectors.	Design of safer new generation (self-inactivating, SIN) vectors, addition of suicide genes. Use of non-integrating systems (electroporation).

technology is slightly different from that used for chimeric monoclonal antibody-derived artificial receptors, the concept remains the same.<sup>43</sup> Patients affected by metastatic melanoma were treated with autologous T cells expressing an anti-MART-1 artificial TCR. Two patients treated out of 15 showed sustained levels of circulating engineered T cells following objective regression of hepatic and pulmonary metastatic lesions.<sup>44</sup>

#### **Limitations and advances in designing ChTCR**

Besides showing and corroborating the strength of ChTCR technology, the available *in vitro* and *in vivo* data also highlight the presence of specific drawbacks, which must be investigated in order to find solutions that ensure better *in vivo* results in the human cancer setting, whilst limiting any possible side effect.

Limitations of ChTCR involve both clinical and technical issues. As far as concerns clinical limitations, the currently used ChTCR lack sufficient specificity for exclusive tumor antigens. This aspect is particularly evident for the CD19 antigen, whose targeting will be accompanied by elimination of normal B cells constitutively expressing this B-lymphoid associated surface molecule. The use of anti-CD19 ChTCR-redirectioned T cells in human subjects will, therefore, likely determine a depression of humoral antibody-mediated immunity, which would put any treated cancer patient at further risk and necessitate continuous supplementation of immunoglobulins.<sup>16</sup> Similar considerations are valid for the CD33 antigen, whose expression is extended to various stages of myeloid-lineage differentiation. Therefore, its targeting could potentially be followed by the killing of bone marrow neutrophil progenitors, with increased risk of bacterial and fungal infections. For these reasons, new lines of research are already exploring more tumor-specific surface antigens, with virtually absent expression on normal tissues.<sup>16</sup>

As far as concerns the technical weaknesses of current ChTCR, different factors must be taken in account,

as summarized in Table 1. The first issue that could easily explain the limited survival of injected ChTCR-expressing cells is the presence of foreign molecules in the chimera (mostly mouse proteins), which induce an immune host response.<sup>14,15</sup> New generation ChTCR will contain entirely humanized proteins, thus avoiding any recognition by the immune system.<sup>45</sup> If this limitation seems likely to be resolved rapidly by technical improvements already available, the definition of the correct activation cascade that must be included in the ChTCR structure to have a full and long-lasting activation of manipulated T cells is more complex. It seems clear, in fact, that the activation following engagement of naïve TCR gives rise to a more complex and powerful activation of T cells, which cannot be totally provided by the existing ChTCR.<sup>46</sup> In line with these considerations, the role of co-stimulation appears to be crucial. Second generation ChTCR all contain a more complex signaling domain, which contains different endodomains with co-stimulatory capacity (CD28, ICOS, CD134 or CD137) (Figure 1).<sup>12-16</sup> Such improved molecules induce a better activation of manipulated target cells, with a higher rate of proliferation, higher level of secretion of interleukin-2 and prolonged survival. The signaling characteristics of ChTCR can be further improved by linking *in cis* more than one co-stimulatory domain or a combination of co-stimulatory and co-receptor domains to the TCR- $\zeta$  chain. Linking the CD28 with the OX40 domain has been shown to greatly enhance the functions of ChTCR-transduced cells with markedly increased proliferation, cytokine release and effector function.<sup>32</sup> Moreover the combination of  $\zeta$ -chain together with co-receptor (*lck*) and co-stimulatory (CD28) signals in a single receptor has been demonstrated to maximize ChTCR sensitivity and potency.<sup>47,48</sup>

A different strategy to guarantee an improved and totally physiological activation of the T cells is to take advantage of the presence of an activated native TCR, transducing CTL which are already specific for a viral

target (the so-called *dual-specific* T cells, as mentioned before).<sup>27,49,50</sup> In this condition the activation of the natural TCR should provide a powerful stimulus, maintaining the killing capacity, the proliferative activity and the durable persistence of the ChTCR-related functions, all present in the same cell. Brenner's group recently adopted EBV-specific CTL, transducing them with a ChTCR targeting the G(D2a) antigen expressed by neuroblastoma cells.<sup>51</sup> Such manipulated cells preserve intact activity towards the viral target and also kill the neuroblastoma cells through the artificial TCR, secreting higher levels of interleukin-2 and proliferating more consistently and durably. This strategy is currently being tested in a phase 1 trial for high-risk neuroblastoma patients. Other investigators have evaluated similar approaches using CMV-specific CTL<sup>52</sup> or influenza virus-specific CTL.<sup>27</sup>

One of the most delicate and discussed topics of any gene transfer approach remains the theoretical chance of inducing oncogenic mutations after DNA integration of the vector. In 2002 Alain Fischer's group reported the occurrence of two cases of T-cell acute lymphoblastic leukemia in two children previously treated with gene-manipulated stem cells for correction of their X-linked SCID immunodeficiency.<sup>53-55</sup> In both cases an inappropriate activation of LMO-2 transcription factor was detected due to proximal insertion of the missing immune gene by the retroviral vector. As thoroughly discussed by Dotti *et al.* in their review,<sup>16</sup> even though this serious adverse event triggered a sort of innate fear for any approach using any viral vector for the correction of any kind of human disease in the entire scientific community, nevertheless this was the only serious event that has occurred so far in more than 40 human trials that have used integrating vectors, and may likely be explained by the peculiarity of the targeted disease (the SCID immunodeficiency) and the type of cells which were genetically manipulated (hematopoietic stem cells).<sup>56,57</sup> In fact, considerable amounts of stem cells were manipulated with the aim of guaranteeing the correction of the immune deficit. Moreover, the transduced T cells, expressing the inserted gene, had a proliferative advantage over the unmanipulated cells. On the other hand, in all the trials that adopted gene transfer of mature T lymphocytes, no malignant transformation has ever been observed, primarily because such mature cells have a limited proliferative capacity and have already reached a stage of terminal differentiation. Obviously, this does not mean that safety considerations regarding the use of integrating vectors are not important, especially when considering (such as for transduced ChTCR-cells) that gene transfer of co-stimulatory molecules or growth-promoting molecules may improve the survival and increase the proliferation rate of gene-manipulated T cells. This automatically implies

that efficient systems of suicide genes are welcome, because they constitute a back-up protection tool, which may be used in the case of unwanted proliferation.<sup>58</sup>

### **Concluding remarks: new challenges for the near future**

The above-mentioned considerations on redirecting cytotoxic T cells towards leukemia/lymphoma-associated antigens show clearly how fascinating ChTCR technology is, but also demonstrate that complex obstacles still bar the way to an effective therapy in the context of leukemias. Only a collaborative multi-center study can provide the opportunity to study the mechanisms underlying the actual limitations of this approach (both *in vitro* and *in vivo*) and to translate this kind of therapy *from bench to bed-side* in the context of phase 1 trials for acute lymphoid and myeloid leukemias and lymphomas. A consortium has been recently founded thanks to funds from the European Community (STREP, Specific Targeted Research or Innovation Project, Sixth Framework Programme), which connects different centers, physicians and researchers from all over Europe. The name of the project is *Childhope* ([www.childhope.eu](http://www.childhope.eu)). The main goal of this collaborative study is to make human T cells that express ChTCR specific for the CD19 and the CD33 antigens, for lymphoid and myeloid malignancies, respectively. These vectors will be used to transduce different types of cell targets (*dual-specific* EBV-CTL, CIK cells,  $\gamma$ - $\delta$  T cells), with the aim of comparing their *in vitro* and *in vivo* efficacy and finally choosing the more potent and persistent stimulation on the artificial receptor towards the selected tumor antigen. After an initial phase, whose only scope will be to validate the potency of the manipulated cells *in vitro* and *in vivo* in animal models, the consortium will assess the safety and potency of the ChTCR-expressing T cells specific for CD19 or CD33 in phase I studies conducted in children with relapsed or refractory B-cell lymphoid leukemias, non-Hodgkin's lymphomas, or acute myeloid leukemias, respectively. With the aim of improving the safety profile of gene transfer, the consortium will evaluate alternative methods of T-cell gene transfer, such as large-scale electroporation of T cells with mRNA (as a means to transduce T cells transiently),<sup>59</sup> in comparison with self-inactivating vectors (SIN), which are characterized by a different pattern of DNA integration able to give a better safety profile.<sup>60</sup> Moreover, always with the aim of maintaining a high efficiency, but concomitantly guaranteeing a way to control any possible *in vivo* unwanted T-cell proliferation, a suicide gene system will be adopted and different combinations will be tested. In particular, attention will be focused on the chRec-iCasp9 suicide system, whose efficiency has been recently demonstrated.<sup>61</sup>

In conclusion, even though the *clinical facts* of leukemia immunotherapy are few and many difficulties stand in the way of translating any experimental approach into clinical intervention,<sup>62</sup> the ChTCR strategy represents an attractive targeted weapon against leukemias. Technical and safety improvements are still required, and phase 1 trials will be carried out in the near future to show the feasibility of their clinical translation and, it is to be hoped, give preliminary indications about their anti-tumor efficacy.

#### Author Contributions

*EB and VM analysed the literature and wrote the manuscript; ED, GMPGA and AB were responsible for critical revision of the text.*

#### Conflict of Interest

*The authors reported no potential conflicts of interest.*

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