

# Immunotherapy for B-Cell Neoplasms using T Cells expressing Chimeric Antigen Receptors

## From antigen choice to clinical implementation

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## العلاج المناعي لأورام الخلايا (B) باستخدام الخلايا (T) المبلورة لمستقبلات هجينة مولدة للمضادات من مرحلة اختيار مولد المضاد إلى التطبيقات السريرية

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**الملخص:** يتم تقييم العلاج المناعي باستخدام الخلايا (T) المبلورة لمستقبلات هجينة مولدة للمضادات كعلاج محتمل لأورام الخلايا (B). أظهرت التجارب السريرية الأخيرة نتائج واعدة. وكلما زاد عدد المضادات المحتملة للترشح، أصبح اختيار المضاد المناسب أكثر تحدياً لتصميم الدراسات ولتقييم أمثل لفعالية الخلايا (T) المبلورة للمستقبلات الهجينة. المطلوب تقييم دقيق للمضادات للتأكد من أن الخلايا (T) المبلورة للمستقبلات الهجينة سوف تقضي على الخلايا الخبيثة بشكل تفضيلي مع ضمان أدنى مستوى سمية ضد الأنسجة الطبيعية. تُبلور الخلايا (B) سطحا محددًا لمولدات المضادات التي يمكن أن تستعمل نظرياً كأهداف لتصميم الخلايا (T) المبلورة للمستقبلات الهجينة. على الرغم من أن العديد من هذه المضادات يمكن أن تحفز استجابات خلوية مناعية فعالة داخل الجسم الحي، فإن تطبيقاتها السريرية ماتزال تمثل تحدياً إذ إن المضادات الوحيدة التي تم اختبارها في التطبيقات السريرية هي (CD19) و (CD20). نستعرض هنا مولدات المضادات للخلايا (B) لتصميم المستقبلات الهجينة كما نذكر العقبات التي يمكن أن تتخلل عملية التعرف على الأهداف الخلوية التي يمكن أن تكون مفيدة.

**مفتاح الكلمات:** العلاج المناعي، خلايا (B)، خلايا (T)، الأورام، المستقبلات الهجينة المولدة للمضادات.

**ABSTRACT:** Immunotherapy with T cells expressing chimeric antigen receptors (CAR) is being evaluated as a potential treatment for B-cell neoplasms. In recent clinical trials it has shown promising results. As the number of potential candidate antigens expands, the choice of suitable target antigens becomes more challenging to design studies and to assess optimal efficacy of CAR. Careful evaluation of candidate target antigens is required to ensure that T cells expressing CAR will preferentially kill malignant cells with a minimal toxicity against normal tissues. B cells express specific surface antigens that can theoretically act as targets for CAR design. Although many of these antigens can stimulate effective cellular immune responses *in vivo*, their implementation in clinical settings remains a challenge. Only targeted B-cell antigens CD19 and CD20 have been tested in clinical trials. This article reviews exploitable B cell surface antigens for CAR design and examines obstacles that could interfere with the identification of potentially useful cellular targets.

**Keywords:** Immunotherapy; B cells; T cells; Neoplasms; Chimeric antigen receptors.

**I**N THE PAST TEN YEARS, MUCH PROGRESS HAS been made in enlarging the treatment options for B-cell neoplasms due mainly to surface targeted therapies. Particularly, the greatest impact has been made with monoclonal antibodies (mAbs), which bind therapeutically to the relevant target antigens causing cell death via several mechanisms. Given their success in treating refractory or

relapsed B-cell neoplasms, mAbs are now included in first-line therapy during the induction phase or intensification period, as well as during maintenance therapy in the post-remission phase of treatment.<sup>1,2</sup> However, as for chemotherapeutic drugs, resistance to monoclonal antibody therapy has emerged as a limiting factor for the effectiveness of these compounds in the treatment of B-cell neoplasms. To

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overcome these limitations, a variety of approaches are being developed to augment the ways by which antibodies kill malignant cells.<sup>3</sup>

An interesting approach to enforce tumour antigen recognition is to generate genetically engineered T cells to express highly active chimeric antigen receptors (CAR) to kill leukaemic cells without the requirement for restriction of the major histocompatibility complex (MHC) molecules. These genetically engineered T-cell constructs combine the antibody-derived antigen-binding motif, known as a single-chain fragment of variable regions (scFv) that recognises the target antigen, with the intracellular functional domains of T-cell receptor (TCR)-related signalling molecules, the CD3 $\zeta$ -chain, and T-cell costimulatory molecules such as CD28, CD134, and CD137.<sup>4,5</sup> Using lentiviral transduction or electroporation, the genetic constructs are introduced into the autologous peripheral T cells collected by leukapheresis procedures [Figure 1]. When expressed on T cells, CAR redirects T-cell specificity and cytotoxic immunoreactivity against the cells expressing the targeted antigen epitope.<sup>6-8</sup> Generally, before an infusion of genetically engineered T cells, patients receive lymphodepleting chemotherapy to eliminate immunosuppressive cells and other lymphoid immune cells that produce growth factors such as interleukin-7 (IL-7) and IL-15.<sup>9,10</sup> Current protocols suggest that lymphodepletion is required to enhance the clinical efficacy of genetically engineered T cells.<sup>11-13</sup> It has been reported that the day of adoptive T-cell transfers in relation to preconditioning regimens is critical as T cells given at day two after stem cell transplantation show superior persistence as compared to those given in later days.<sup>14</sup>

Until recently, development of this personalised T-cell-based therapy was limited due to the lack of gene transfer techniques and efficient T-cell culture systems. Therefore, previous clinical applications with T cells expressing CAR showed modest clinical activities, as these engineered T cells demonstrated a limited expansion *in vivo* and rapidly disappeared from circulation.<sup>6,7,15,16</sup> With the most recent wave of genomic technology, great progress has been made in improving gene transfer technology and in developing methods to enhance T-cell effector functions. These advances have made possible the creation of second- and third-generation T cells

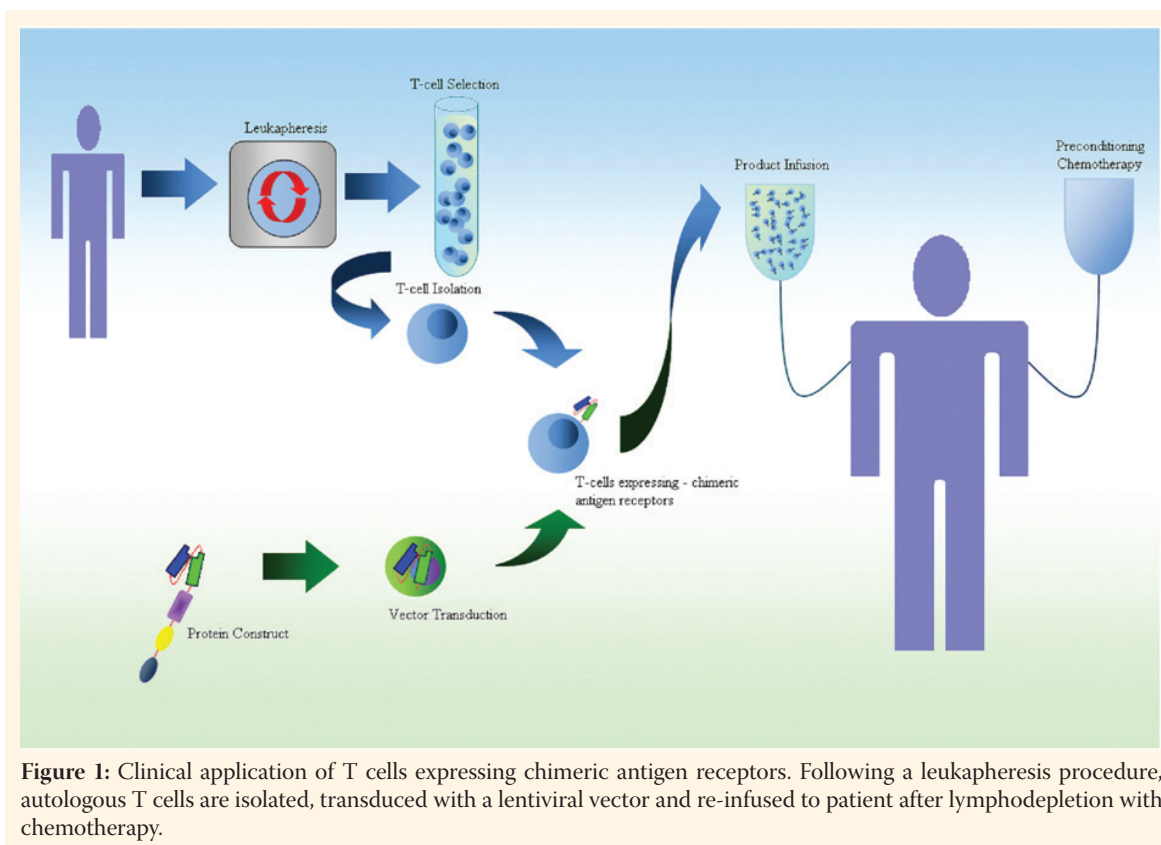
expressing CAR, which are endowed with potent effector functions.<sup>4-8</sup> Thus, several groups have evaluated these second generations of CAR as a potential treatment to kill leukaemic cells in patients with B-cell neoplasms.<sup>17-22</sup>

As for any targeted immunotherapy, the success of novel genetically engineered T cells relies heavily on the characteristics of the target cell surface antigen. Since the list of potential candidate antigens is expanding, the choice of a suitable target becomes more crucial for both the design and optimal efficacy of CAR. In addition, a critical evaluation of candidate target antigens is required to ensure that T cells expressing CAR will preferentially kill malignant cells with little toxicity against normal tissues. Therefore, this article will briefly review the B-cell surface antigens, which may be exploited as targets for CAR design to treat B-cell neoplasms. It will also examine potential obstacles that could interfere with the identification of these cellular targets, which might be useful for CAR immunotherapy.

## Characteristics of an Ideal Target Antigen

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An important step in designing a CAR is the selection of a good candidate antigen. The target antigen should possess certain characteristics to justify scientifically its clinical applicability. First, the suitable antigen should ideally be expressed by malignant cells but not on normal cells. While this seems to be a reasonable criterion for antigen selection, it may not be a prerequisite in every case, especially for B-cell neoplasms where most potential targets are also expressed by some cells of normal tissues. In these cases, the target antigen should be expressed in higher amounts on malignant cells relative to normal cells. Second, the target antigen should be expressed on the surface of the malignant cells. This reasonable cell surface expression is important to avoid immune escape of malignant cells, which are endowed with a strong predilection to down-regulate antigen expression.<sup>23</sup> Third, beside its immunogenicity, the target antigen should not be internalised by malignant cells to ensure the induction of immune effector cell functions. Fourth, an ideal antigen should exhibit negligible shedding and only a small amount of secretion with a limited potential for mutation to avoid compromising its



**Figure 1:** Clinical application of T cells expressing chimeric antigen receptors. Following a leukapheresis procedure, autologous T cells are isolated, transduced with a lentiviral vector and re-infused to patient after lymphodepletion with chemotherapy.

therapeutic utility. Finally, the antigen of interest should preferentially be involved in key functions of the target cell such as growth-regulating signalling, immune recognition, or apoptosis.

## Potential Candidate Antigens for CAR Design in B-Cell Neoplasms

B cells express a variety of specific surface antigens that can theoretically act as targets for CAR design. Although many of these antigens showed the ability to stimulate effective cellular immune responses *in vivo*, their implementation in clinical settings remains a big challenge. The ones with the most promise in B-cell neoplasms are summarised in Figure 2, and their characteristics are briefly described in Table 1.

### CD19 ANTIGEN

Because of its promising properties in B-cell development, pioneering work in CAR design focused on the CD19 antigen. This molecule was first identified as the B4 antigen on normal and malignant human B lymphocytes.<sup>24</sup> This 95-kDa type I transmembrane glycoprotein is a cell

surface molecule exclusively expressed by B cells and follicular dendritic cells among cells of the hematopoietic system.<sup>25,26</sup> Of interest, its expression is lost during terminal B-cell differentiation into plasma cells. CD19 is involved in growth regulation of B cells through the B-cell antigen receptor. It is also required for the development of marginal zone B cells, germinal centre formation, T-dependent antibody response, and B-cell memory maintenance.<sup>26</sup> Compared to other B-cell surface molecules, CD19 is expressed at high levels on essentially all B-lineage leukaemias and lymphomas, making this antigen an excellent tumour-associated antigen for CAR development.

### CD20 ANTIGEN

Following the identification of surface immunoglobulin, CD20 was the first cell surface differentiation antigen of human B cells to be identified by a monoclonal antibody. CD20 is a 33-37-kDa integral membrane protein with four transmembrane domains and an extracellular disulfide bond.<sup>27</sup> It is expressed on pre-B and mature naïve B cells but not on plasma cells or early pro-B cells. At the primary sequence level, CD20 shows little sequence homology with CD19 but

**Table 1:** Characteristic features of potential target B-cell antigens for chimeric antigen receptor design.

Potential Antigens	Structural Features	B-Cell Expression	In vitro and in vivo Studies	Clinical Trials Usage	References
CD19	95-kDa glycoprotein	All B cells except plasma cells	Raji and Daudi B cell lines SCID/beige mice NOD/SCID mice	CLL FL SMZL B-ALL	18, 19, 20, 21, 22
CD20	33-37-kDa non-glycosylated phosphoprotein	All B cells except plasma cells and early pro-B cells	MB185 cell line SCID/beige mice Balb/c mice	MCL FL	17
CD22	135-140-kDa sialoglycoprotein	All B cells except plasma cells and memory B cells	Raji, Daudi and Ramos B cell lines	ND	57
CD23	45-kDa glycoprotein	All B cells except immature bone marrow B cells	MEC-1 CLL, BJAB and Jeko-1 cell lines Rag2 mice	ND	58
CD30	102-120-kDa glycoprotein	Activated B cells	ND	ND	37
CD40	54-50-kDa glycosylated phosphoprotein	All stages of B-cell development	ND	ND	39
CD52	21-28kDa glycoprotein	All B cells	ND	ND	41
CD70	50-kDa glycoprotein	Activated B cells	Raji, Daudi, CLL-120, 239T, U266 and K562 cell lines SCID mice	ND	59
CD74	31-41-kDa glycoprotein	All B cells	ND	ND	44
CD80	60-kDa glycoprotein	Activated B cells	ND	ND	47
Igk light chain	25-kDa glycoprotein	All B cells	Raji, Daudi, BJAB CLL-120, SP-53 and K562 cell lines SCID mice	ND	60

*Legend: Raji, Daudi and Ramos = Human Burkitt's lymphoma cell line; SCID = severe combined immunodeficient; NOD = non-obese diabetic; MB185 = mouse pre-B cell lymphoma cell line; MEC-1 CLL and CLL-120 = chronic lymphocytic leukemia cell line; BLAB = B-lymphoblastoid cell line; JeKo-1 = mantle cell lymphoma cell line; 293T = human embryonic kidney cell line; U266 = myeloma cell line; K562 = human erythroleukaemic cell line; SP-53 = lymphoma cell line; CLL = chronic lymphocytic leukaemia; FL = follicular lymphoma; SMZL = splenic marginal zone lymphoma; B-ALL = B-cell acute lymphoblastic leukaemia; MCL = mantle cell lymphoma; ND = not determined.*

both antigens interact with MHC class II molecules on B cells. Current studies suggest that CD20 is a component of a signal transduction complex that is involved in the growth regulation of B lymphocytes following activation.<sup>28</sup> In addition to its restricted B-lineage expression, CD20 is present on the majority of B-cell lymphomas, providing a strong rationale for CAR design.

## CD22 ANTIGEN

The CD22 antigen was originally identified as a B-cell-associated adhesion molecule that appears to function in the regulation of B-cell activation. It is a member of the sialic-acid binding immunoglobulin-like lectin (SIGLEC) family. SIGLEC proteins are a subset of the immunoglobulin super-family of

cell recognition molecules that bind to sialic acid-containing glycans of the cell surface.<sup>29</sup> CD22 is a 135-140-kDa B-cell-specific transmembrane sialoglycoprotein, expressed on all B cells except on memory B cells and plasma cells.<sup>30</sup> It regulates multiple B-cell functions including the cellular activation thresholds and survival. In this role, CD22 modulates B-cell dependent immune responses, prevents autoimmunity, and controls the homing of B cells back to the bone marrow. It also modulates T-cell signalling upon binding to its ligand on T cells.<sup>31</sup> CD22 is strongly expressed by many B-cell lymphomas and leukaemias, particularly hairy cell leukaemia, where it is universally highly expressed.<sup>32</sup> Given that CD22 is found on normal B cells at low levels but not on plasma cells, these

characteristics make CD22 an attractive target for CAR development.

## CD23 ANTIGEN

The CD23 antigen was initially defined as the low-affinity receptor for immunoglobulin (Ig)-E, also known as FcεRII. It is a 45-kDa type II transmembrane glycoprotein expressed on B cells but not on immature bone marrow B lymphocytes. Its expression was also reported on various cell types including monocytes, eosinophils, and platelets.<sup>33</sup> CD23 participates in multiple regulatory functions either as a membrane-bound glycoprotein or as a freely soluble protein. Among these functions, CD23 regulates the production of immunoglobulin-E, the antibody that mediates the allergic reactions, and B-cell growth, as underlined by its overexpression and abnormal regulation in two B-cell neoplasms resulting from the clonal expansion of mature B cells (i.e. chronic lymphocytic leukaemia and small cell lymphocytic lymphoma).<sup>34</sup> In addition, the Epstein-Barr virus (EBV)-transformed B cells express high levels of CD23 and its expression is used to delineate classical Hodgkin's lymphoma from mediastinal diffuse large lymphoma.<sup>34</sup> In combination, these properties support CD23 credibility as an interesting target for CAR design in B-cell neoplasms.

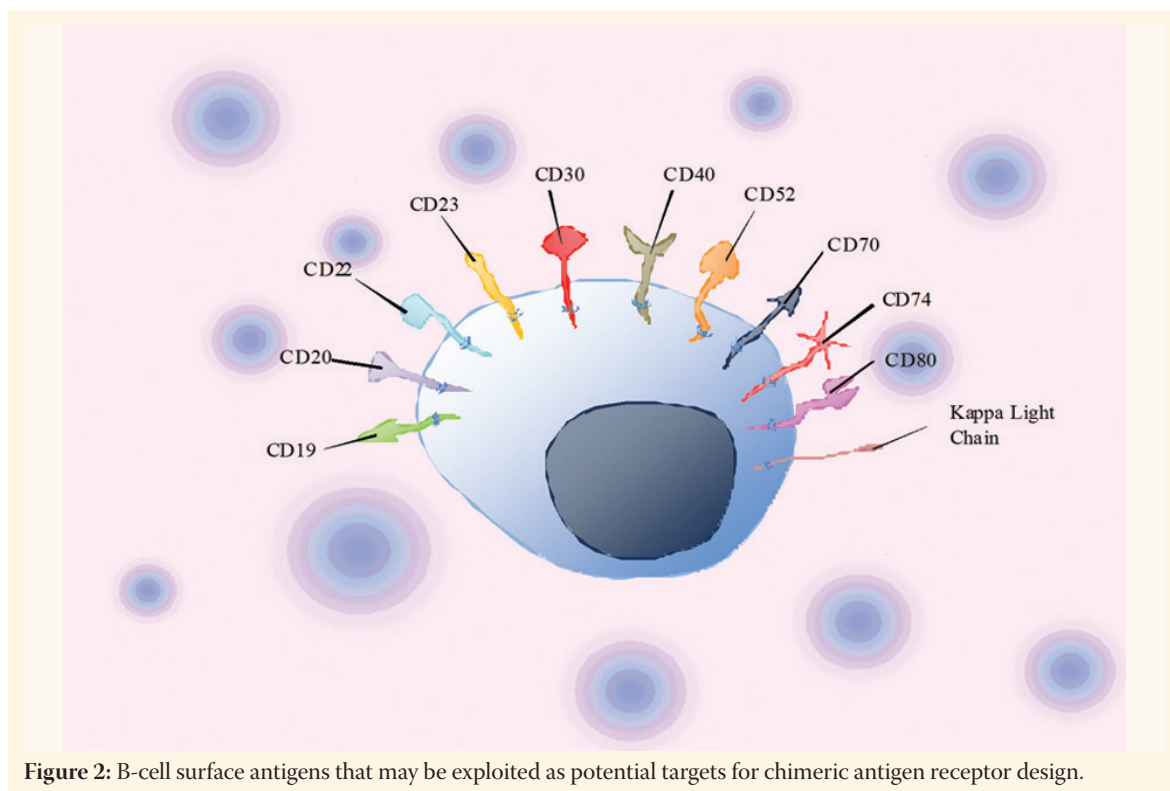
## CD30 ANTIGEN

Based on its presence on Reed-Sternberg cells, which are characteristic of Hodgkin's lymphoma, CD30 was identified by means of the monoclonal antibody Ki-1 in 1982. Since then, CD30 has been used as a diagnostic marker for Hodgkin's lymphoma and allowed the description of a new group of malignant disorders named CD30-positive lymphoproliferative disorders.<sup>35</sup> The CD30 antigen is a member of the tumour necrosis factor receptor (TNFR) superfamily which includes CD40, CD70, CD95, and CD134, among others.<sup>36</sup> CD30 is a type I glycoprotein with a molecular weight of 102-120-kDa. Structurally, an 85-kDa form, which is a product of the proteolytic cleavage of the CD30 antigen, could be detected in the blood of patients with CD30-positive lymphoproliferative disorders or autoimmune diseases.<sup>37</sup> Under normal conditions, CD30 expression is detected in activated B, T and natural killer cells. In addition, lower levels of expression were reported

on activated monocytes and eosinophils. The expression of CD30 is highly induced on B cells by mitogen activators, or as a result of viral infection with human T-cell lymphotropic viruses (HTLV), human immunodeficiency virus (HIV), or EBV.<sup>37</sup> Of interest is the finding that CD30 expression is absent on most cells outside the immune system. Interaction of CD30 with its ligand CD153 induces the recruitment of signalling proteins, which leads to the transduction of several biological signals such as cell death, proliferation, activation, and differentiation depending on the cellular context.<sup>37</sup> In addition to the malignant disorders cited above, CD30 is also expressed on 20% of B-cell lymphomas and some multiple myeloma cells. Thus, because of its restricted profile expression in normal conditions, the CD30 antigen appears to be an attractive target for CAR development.

## CD40 ANTIGEN

Like CD30, the CD40 antigen belongs to the TNFR superfamily and is a surface receptor of 45-50-kDa, playing a central role in T-cell-dependent B-cell activation and proliferation<sup>36</sup> CD40 is expressed during all stages of B-cell development and differentiation, whereas its ligand (CD40L or CD154) is mainly expressed on activated T cells. In addition to its expression on all antigen-presenting cells such as dendritic cells, follicular dendritic cells, and monocytes, CD40 is also expressed on non-haematopoietic cells including endothelial cells, fibroblasts, and epithelial cells.<sup>38</sup> In fact, CD40 is involved in the amplification and regulation of inflammatory responses by providing critical signals required for the optimal activation of immune cells. These key signals resulted in optimal antigen presentation, expansion of antigen-specific memory and effector T cells, proliferation of B-cell responses, immunoglobulin class switching, and secretion of cytokines.<sup>39</sup> Currently, the CD40-CD40L immunostimulatory effect is used to enhance antigen presentation in gene transfer protocols. High levels of CD40 expression were detected on a wide range of malignant cells including B-cell neoplasms, bladder cells, and carcinoma cell types, among others. It has been reported that in Hodgkin's lymphoma, hairy cell leukaemia, chronic lymphocytic leukaemia, and follicular lymphomas, CD40 activation contributed to tumour survival and resistance to chemotherapy. In HIV-related



**Figure 2:** B-cell surface antigens that may be exploited as potential targets for chimeric antigen receptor design.

lymphomas, CD40 binding triggered the growth of B cells via the activation of the nuclear factor-kappa-B (NF- $\kappa$ B) pathway that in turn stimulated HIV replication.<sup>38,39</sup> Because of the key role of CD40 in mediating growth of B-cell neoplasms, this antigen could be a promising target for CAR development.

### CD52 ANTIGEN

The CD52 antigen, also known as the Cambridge pathology (CAMPATH)-1 antigen, is a surface glycoprotein with a molecular weight of 21-28-kDa. CD52 comprises a short peptide sequence of twelve amino acids and a large complex N-linked oligosaccharide, and is attached to the cellular membrane by a glycosylphosphatidylinositol (GPI) anchor.<sup>40</sup> It is abundantly expressed on virtually all B and T lymphocytes and, to a lesser degree, on monocytes and natural killer cells. It is also expressed on a large proportion of lymphoid B-cell malignancies including lymphomas and chronic lymphocytic leukaemia.<sup>41</sup> The response of B lymphocytes, following its activation via the CD52 antigen, varies according to both the state of cellular maturation and the way the antigen is presented to cells and results in clonal expansion, differentiation, anergy, or deletion by apoptosis. It has been reported that the cross-linking of CD52 on

B-cell lines resulted in profound growth inhibition, corroborating the notion that this antigen might be used as a target for CAR design.

### CD70 ANTIGEN

The CD70 antigen is a member of the TNFR superfamily, like the CD30 and CD40 antigens.<sup>36</sup> CD70 is a type II transmembrane glycoprotein with a molecular weight of 50-kDa. It is transiently expressed by activated T and B lymphocytes, monocytes, and dendritic cells. It is also expressed on stromal thymic cells of the medulla, but not on other normal cells or tissues.<sup>42</sup> The interaction of CD70 with its ligand CD27 regulates the expansion and differentiation of memory and effector T cells whereas in B cells, CD70-CD27 co-stimulation promotes B-cell expansion through several signalling pathways including spleen tyrosine kinase, phosphoinositide 3-kinase, and NF- $\kappa$ B.<sup>43</sup> Of interest, CD70 expression is documented in many different types of lymphomas, leukaemias, and carcinomas. CD70 is abundantly expressed by B-cell derived non-Hodgkin lymphoma including diffuse large B-cell lymphoma, follicular lymphoma, Burkitt's and mantle cell lymphomas, and B-cell lymphocytic leukaemia as well as Waldenström macroglobulinaemia, multiple myeloma, and chronic lymphocytic leukaemia.<sup>42</sup> It is also highly

expressed on Reed-Sternberg cells in Hodgkin's lymphoma and other non-lymphoid tumour cells such as nasopharyngeal carcinoma, astrocytoma, glioblastoma, and renal cell carcinoma (RCC) as well as in EBV- and HTLV-associated malignancies.<sup>42</sup> Given the differential expression pattern of CD70 in leukaemic cells as compared to normal B cells, this antigen represents a prominent target for CAR development.

## CD74 ANTIGEN

The CD74 antigen, also known as an invariant chain (Ii), is a conserved type II membrane glycoprotein with a molecular weight of 31-41-kDa. The Ii on the cell surface is present in four isoforms and controls several aspects of the immune system.<sup>44</sup> In addition to its role as a chaperone molecule for MHC class II molecules, CD74 is involved in several signalling pathways. CD74 is fundamental to the correct folding and transport of MHC class II molecules to endosomes and lysosomes as well as in antigen loading. It is directly involved in the maturation of B cells through the NF- $\kappa$ B pathway.<sup>44</sup> It also serves as the high-affinity receptor for the pro-inflammatory cytokine known as the macrophage migration inhibitory factor, which activates innate immune cells such as macrophages and monocytes through kinase signalling pathways.<sup>44</sup> Under normal conditions, CD74 is expressed on MHC class II positive cells such as B cells, monocytes, macrophages, thymic epithelium, Langerhans cells, activated T cells and dendritic cells. Under the inflammatory processes, CD74 is also detected in high levels on endothelial and epithelial cells as well as on a variety of malignant cells.<sup>45</sup> Its over-expression is observed in several forms of cancer and many B-cell neoplasms, including non-Hodgkin lymphoma, chronic lymphocytic leukaemia, multiple myeloma, and follicular lymphoma. In many of these cancers, increased CD74 expression is associated with tumour progression and poor clinical outcomes.<sup>45</sup> Compared to normal tissue, the relatively high expression of CD74 combined with the rapid internalisation of CD74 on ligation makes it an attractive target for CAR design.

## CD80 ANTIGEN

Among other potential antigens for CAR design is CD80, a 60-kDa transmembrane glycoprotein. This antigen is a member of the B-family that

includes CD80-, CD86-, B7-H3, B7-H4, the inducible costimulator ligand, and the programmed death-1 and death-2 ligands.<sup>46</sup> These molecules are transmembrane, or glycosyl-phosphatidylinositol (GPI)-linked proteins, playing key roles in immune responses by providing co-stimulatory or co-inhibitory signals upon binding their receptor. In addition to its well-known role in regulating T-cell activity, recent studies suggest that CD80 also regulates B-cell functions.<sup>47</sup> Cross-linking of CD80 on lymphoma cell lines resulted in growth inhibition and up-regulation of pro-apoptotic molecules.<sup>48</sup> CD80 is transiently expressed on the surface of activated B cells but is constitutively expressed on a variety of non-Hodgkin lymphomas, including follicular lymphoma, making it a good target for CAR design.

## Immunoglobulin Light Chains

Another potential antigen for CAR design is the kappa ( $\kappa$ ) light chain (LC) of the Ig molecules. Ig is formed by two identical heavy chains and two identical LCs, lambda ( $\lambda$ ) and  $\kappa$ . B lymphocytes express surface monoclonal Igs with either  $\kappa$  or  $\lambda$  light chains. Since expression of  $\lambda/\kappa$  LCs is clonally restricted, and B-cell neoplasms such as low-grade non-Hodgkin lymphoma and chronic lymphocytic leukaemia are themselves clonal, the malignant cells in a given patient will express either  $\kappa$  or  $\lambda$  LC.<sup>49</sup> Therefore, targeting the LC expressed by malignant B-cells should spare the normal population of B lymphocytes that express the reciprocal LC. Thus, LC  $\lambda$  or  $\kappa$  may be considered an attractive antigen for CAR development.

## Improving the Clinical Applicability of Targeted Antigens for Designing CAR

As discussed above, several candidate antigens can be targeted for designing CAR. These antigens are typically normal cell constituents that are over-expressed in B-cell neoplasms. The potential contribution of each antigen to CAR development and implementation is still debated. For instance, what determines which antigens should be first targeted? An additional question

is which B-cell neoplasms should be studied first? In this regard, defining shared features of target antigens is of paramount importance in designing and implementing CAR in B-cell neoplasms. CAR targeting CD19 (CART19) and CD20 (CART20) antigens were the first molecules to be used in B-cell malignancies. In early *ex vivo* studies, both T cells expressing CART19 (CART19) and CART20 efficiently lysed a wide panel of tumour cell lines expressing CD19 and CD20 antigens, respectively, as well as B cells freshly isolated from patients with B-cell neoplasms.<sup>50–56</sup> In animal models, CART19 and CART20 also eradicated systemic cell tumours established in severe combined immunodeficient (SCID) mice that were expressing CD19 or CD20.<sup>51,52</sup> These preclinical experiments have supported the notion that under appropriate conditions, T cells can be therapeutically redirected to eliminate malignant B cells and have justified the translation of these genetically engineered T cells to clinical settings. Recently, in proof of principle studies, adoptive transfer of CART19 or CART20 to patients with chemotherapy-refractory chronic lymphocytic leukaemia or follicular lymphoma demonstrate encouraging results in terms of reducing the leukaemic B-cell burden.<sup>17–22</sup> Taken together, CART19- and CART20-based clinical trials are at the advanced planning stage in several centres around the world, and the results of these will be of importance to further establish the clinical efficacy of this therapeutic approach. Other CAR-targeting CD22 (CART22), CD23 (CART23), and CD70 (CART70) antigens as well as  $\kappa$  LC (CART $\kappa$ ) were also developed showing promising results in *in vitro* and *in vivo* mouse tumour models.<sup>57–60</sup>

With respect to the therapeutic potential of tested CAR, it is worthwhile to note that suboptimal cytotoxic effects of these genetically engineered T cells vary among targeted antigens. Although several explanations may be proposed, one possible reason is the difference in expression levels of the targeted antigens in malignant and normal B cells. For example, it has been reported that normal B cells express high levels of CD20 when compared to CD22, with copy numbers per cell of approximately  $150 \times 10^3$  and  $30 \times 10^3$ , respectively.<sup>61,62</sup> This high antigen intensity of CD20 on B cells may lead to anergy or deletion of engineered T cells, as demonstrated in adoptively transferred antigen-specific T cells in transgenic mice.<sup>63</sup> Malignant B

cells also showed a substantial variability over time in the expression intensity of different targeted antigens. The expression levels of targeted antigens on B cells may vary according to type of B-cell neoplasms, stage of the disease, and also from patient to patient.<sup>61,62,64</sup> Thus, it is of importance that optimisation of CAR design benefits from the quantification of information about expression levels of targeted antigens on malignant B cells for each patient to increase anti-tumour activities. Therefore, the challenge now is to develop methods that can precisely measure the expression levels of targeted antigens on malignant and normal B cells in an accurate and reproducible way. Additionally, these standardised methods should be suited to clinical settings, easy to run, and non-invasive. One such method is flow cytometry, a robust and well-established technique that is regarded as an excellent immunophenotyping tool, as multiple molecules and surface antigens can be detected in individual cells.<sup>65</sup> At present, it is not known to what extent heterogeneous antigen expression by individual malignant B cells represents a problem for CAR immunotherapy. However, it is quite likely that malignant B cells with low levels of targeted antigens may escape immunotherapy with engineered T cells. Since the targeted antigens are distinct molecules, the possibility exists for combining CART19 or CART20 with CART22, CART23, CART70, or CART $\kappa$  potentially to yield additive or synergistic activities against malignant B cells. In addition, to eliminate completely all malignant B cells, effective immunotherapy with CAR requires targeting surface antigens, which are expressed with great specificity by the earliest leukaemic progenitor. In this regard, engineered T cells should successfully penetrate deeper into the tissue and home to the sites with residual leukaemic B cells. Besides their capacity to proliferate and survive *in vivo*, the efficacy of engineered T cells to eradicate residual leukaemic B cells may also be dependent on the choice of the epitope targeted by the potential antigens. Clearly, the position of the targeted epitope itself, particularly its position within the antigen, has a major impact on the efficacy of engineered T cells activation independent of the scFv binding efficiency.<sup>66</sup>



## Long term Potential Toxicities of Targeted B Cell Antigens

In order to establish T cells expressing CAR as an effective immunotherapy for B-cell neoplasms, several issues still need to be overcome. Some of these issues include the long-term potential toxicity to normal cells, long half-life, potential cross-reactivity, immuno-toxicity, and genotoxicity of CAR.

### TOXICITY TO NORMAL CELLS

An important concern with T cells expressing CAR is the long-term toxicity resulting from the depletion of normal human cells, which are important for homeostatic functions. This toxicity depends mainly on the distribution of the targeted antigens in normal tissues, as recently demonstrated in a clinical trial using T cells expressing CARTG250 (CARTG250), a prominent RCC antigen.<sup>67</sup> In early studies, monoclonal antibodies against this antigen showed a good safety profile in patients with RCC, but when scFv specificity was tested in CARTG250, severe hepatotoxicity occurred due to the reactivity of engineered T cells against the G250 antigen expressed at very low levels on the biliary epithelium. Similarly, based on the widely used humanised mAb trastuzumab in cancer patients, a third generation of T cells expressing CAR was developed against the tumour antigen ERBB2. The genetically engineered T cells displayed a robust anti-tumour activity in animal models, but strongly reacted with the ERBB2 antigen expressed at low levels on lung epithelial cells, resulting in sudden death of the patient five days after treatment.<sup>68</sup> Collectively, these results demonstrate that the safety of targeted antigens with mAbs does not translate into the safety of T cells expressing CAR because these engineered T cells with high-avidity receptors can respond to cells expressing their targeted antigens at levels too low to be detected with conventional means. Although in B cell neoplasms, depletion of normal B cells following an engineered T-cell infusion could be medically managed by Ig transfer and antiviral drugs, the potential risk of a negative impact on other organ functions due to low levels of antigen expression should be carefully evaluated.

### LONG HALF-LIFE

The issue of the long half-life of T cells expressing CAR remains a major problem. Unlike mAbs, which have defined half-lives, engineered T cells may potentially survive and function for years. Therefore, the risks versus benefits should be clearly balanced for any given patient and a given CAR design. As a cautionary note, early therapy with CART19 resulted in the death of a patient who was suffering from chronic lymphocytic leukaemia with a cytokine storm, supporting further the direct cytotoxic potency of these engineered T cells.<sup>69</sup>

### POTENTIAL CROSS-REACTIVITY

The possibility of unwanted cross-reactivity is becoming increasingly important in CAR design. Engineered T cells may react with more than one antigenic determinant if some modifications occur when transfecting these T cells with viral vectors. This cross-reactivity may arise because the targeted antigens share an epitope with other tissue antigens, or because they have an epitope which is structurally similar to one used for designing CAR. It may also result from an epitope or antigen spreading, a process in which engineered T cells may expand their immune responses beyond the primary epitope to target other epitopes on normal cells. While these cross-reactivities are difficult to predict, they may lead to severe toxicity, especially since new targeted antigens are being evaluated with second or third generations of CAR. These latter are endowed with potent cytotoxic activities by killing cells which express just a few antigen molecules.

### POTENTIAL IMMUNOTOXICITY

A key issue for the use of engineered T cells is the potential immunotoxicity of CAR. It has recently been reported that immune responses against both viral vector and scFv binding epitopes can be detected in patients receiving engineered T cells.<sup>70,71</sup> As a consequence, these immune responses may compromise the persistence of engineered T cells and also limit their anti-tumour activities. It is therefore essential to monitor carefully a host's immune responses against all transgene products, including vectors themselves in order to increase the efficacy of engineered T cells.

## POTENTIAL GENOTOXICITY

Insertion of suicide genes into the protein constructs could be envisaged to further limit long-term CAR toxicities. However, introduction of genetic material may lead to genotoxicity; that considered, so far there has been no reported evidence that retrovirally engineered mature T cells induce mutagenesis.<sup>72,73</sup> Since their first description, engineered T cells have been used in more than one hundred patients with no malignant transformation reported in any case, contrasting with what was observed with genetically modified haematopoietic stem cells.<sup>74</sup> To mitigate CAR toxicities, approaches such as a dose-escalating strategy or splitting T-cell doses over two or more days may be applied, particularly when new targeted antigens are first evaluated.<sup>8,75</sup> Another approach is to administer high-dose corticosteroids, which could effectively eliminate engineered T cells as was demonstrated in RCC patients receiving CARTG250.<sup>67</sup>

## Conclusion

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As engineered T-cell designs continue to improve, the selection of therapeutic targeted antigens has become crucial to the development of CAR. Lessons learned so far from pilot clinical trials in patients treated with T cells expressing CAR clearly emphasise the importance of the choice of the right antigen. Intense investigation is underway to discover more candidate antigens not only to expand the repertoire of potential targets for CAR design but also to identify those antigens that will provide selected killing of malignant B cells while sparing normal lymphocytes. A critical issue remains in identifying those targeted antigens that could be specifically expressed on B cells in individual neoplasms but not necessarily be used across all categories of patients with B cell malignancies.

By further understanding the characteristics of potential targeted antigens, it may be possible to identify key epitopes that will help design new CAR with the goal of providing maximal clinical efficacy of engineered T cells. It might also be expected that multiple antigenic epitopes could be combined and targeted to further improve the killing properties of CAR therapy.

Equally important, research should also focus on identifying subsets of patients who are likely

to respond to a specific target antigen. This will provide a rational basis for target antigens to be exploited in well-designed multicentre studies to establish engineered T-cell immunotherapy as a viable option in the treatment of B-cell neoplasms. One might also argue that the selection of T-cell populations that could be used for CAR design, conditioning regimens, and infused T-cell doses may be different for each targeted antigen. Thus, it is quite possible that the only improvements in all these aspects will lead to significant progress that will ultimately advance the field of CAR therapy. This will require collaboration across different academic groups representing different disciplines in order to provide faster information that could be quickly translated into the CAR design. In the meantime, it is hoped that ongoing clinical trials will continue to generate increasing enthusiasm for the application of this personalised T-cell-based immunotherapy in B-cell neoplasms.

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## CONFLICT OF INTEREST

None.

## References

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1. Chamuleau ME, van de Loosdrecht AA, Huijgens PC. Monoclonal antibody therapy in haematological malignancies. *Curr Clin Pharmacol* 2010; 5:148–59.
2. Cartron G, Watier H, Golay J, Solal-Celigny P. From the bench to the bedside: Ways to improve rituximab efficacy. *Blood* 2004; 104:2635–42.
3. Quintás-Cardama A, Wierda W, O'Brien S. Investigational immunotherapeutics for B-cell malignancies. *J Clin Oncol* 2010; 28:884–92.
4. Cartellieri M, Bachmann M, Feldmann A, Bippes C, Stamova S, Wehner R, et al. Chimeric antigen receptor-engineered T-cells for immunotherapy of cancer. *J Biomed Biotechnol* 2010; 2010:956304.
5. Kohn DB, Dotti G, Brentjens R, Savoldo B, Jensen M, Cooper LJ, et al. CARs on track in the clinic. *Mol Ther* 2011; 19:432–8.
6. Jena B, Dotti G, Cooper LJ. Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor. *Blood* 2010; 116:1035–44.
7. Sadelain M, Brentjens R, Rivière I. The promise and potential pitfalls of chimeric antigen receptors. *Curr Opin Immunol* 2009; 21:215–23.

8. Ertl HC, Zaia J, Rosenberg SA, June CH, Dotti G, Kahn J, et al. Considerations for the clinical application of chimeric antigen receptor T cells: Observations from a recombinant DNA Advisory Committee Symposium held June 15, 2010. *Cancer Res* 2011; 71:3175–81.
9. Gattinoni L, Finkelstein SE, Klebanoff CA, Antony PA, Palmer DC, Spiess PJ, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. *J Exp Med* 2005; 202:907–12.
10. Dudley ME, Yang JC, Sherry R, Hughes MS, Royal R, Kammula U, et al. Adoptive cell therapy for patients with metastatic melanoma: evaluation of intensive myeloablative chemoradiation preparative regimens. *J Clin Oncol* 2008; 26:5233–9.
11. Cui Y, Zhang H, Meadors J, Poon R, Guimond M, Mackall CL. Harnessing the physiology of lymphopenia to support adoptive immunotherapy in lymphoreplete hosts. *Blood* 2009; 114:3831–40.
12. Wrzesinski C, Paulos CM, Kaiser A, Muranski P, Palmer DC, Gattinoni L, et al. Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells. *J Immunother* 2010; 33:1–7.
13. Muranski P, Boni A, Wrzesinski C, Citrin DE, Rosenberg SA, Childs R, et al. Increased intensity lymphodepletion and adoptive immunotherapy—how far can we go? *Nat Clin Pract Oncol* 2006; 3:668–81.
14. Rapoport AP, Stadtmauer EA, Aqui N, Vogl D, Chew A, Fang HB, et al. Rapid immune recovery and graft-versus-host disease-like engraftment syndrome following adoptive transfer of costimulated autologous T cells. *Clin Cancer Res* 2009; 15:4499–507.
15. Brocker T, Karjalainen K. Signals through T-cell receptor-zeta chain alone are insufficient to prime resting T lymphocytes. *J Exp Med* 1995; 181:1653–9.
16. Brocker T. Chimeric Fv-zeta or Fv-epsilon receptors are not sufficient to induce activation or cytokine production in peripheral T cells. *Blood* 2000; 96:1999–2001.
17. Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Greisman HA, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood* 2008; 112:2261–71.
18. 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 recognise CD19. *Blood* 2010; 116:4099–102.
19. 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; 365:725–33.
20. Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. *Sci Transl Med* 2011; 3:95ra73.
21. Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. *Blood* 2011; 118:4817–28.
22. Kochenderfer JN, Dudley ME, Feldman SA, Wilson WH, Spaner DE, Maric I, et al. B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells. *Blood* 2012; 119:2709–20.
23. Lollini PL, Forni G. Cancer immunoprevention: Tracking down persistent tumor antigens. *Trends Immunol* 2003; 24:62–6.
24. Nadler LM, Anderson KC, Marti G, Bates M, Park E, Daley JF, et al. B4, a human B lymphocyte-associated antigen expressed on normal, mitogen-activated, and malignant B lymphocytes. *J Immunol* 1983; 131:244–50.
25. Schriever F, Freedman AS, Freeman G, Messner E, Lee G, Daley J, et al. Isolated human follicular dendritic cells display a unique antigenic phenotype. *J Exp Med* 1989; 169:2043–58.
26. Tedder TF. CD19: A promising B cell target for rheumatoid arthritis. *Nat Rev Rheumatol* 2009; 5:572–7.
27. Tedder TF, Engel P. CD20: A regulator of cell-cycle progression of B lymphocytes. *Immunol Today* 1994; 15:450–4.
28. Ernst JA, Li H, Kim HS, Nakamura GR, Yansura DG, Vandlen RL. Isolation and characterisation of the B-cell marker CD20. *Biochemistry* 2005; 44:15150–8.
29. Crocker PR, Paulson JC, Varki A. SIGLECs and their roles in the immune system. *Nat Rev Immunol* 2007; 7:255–66.
30. Tedder TF, Tuscano J, Sato S, Kehrl JH. CD22, a B lymphocyte-specific adhesion molecule that regulates antigen receptor signaling. *Annu Rev Immunol* 1997; 15:481–504.
31. Walker JA, Smith KG. CD22: An inhibitory enigma. *J Immunol* 2008; 123:314–25.
32. Robbins BA, Ellison DJ, Spinosa JC, Carey CA, Lukes RJ, Poppema S, et al. Diagnostic application of two-color flow cytometry in 161 cases of hairy cell leukemia. *Blood* 1993; 82:1277–87.
33. Bonnefoy JY, Lecoanet-Henchoz S, Gauchat JF, Graber P, Aubry JP, Jeannin P, et al. Structure and functions of CD23. *Int Rev Immunol* 1997; 16:113–28.

34. Acharya M, Borland G, Edkins AL, Maclellan LM, Matheson J, Ozanne BW, et al. CD23/FcεRII: Molecular multi-tasking. *Clin Exp Immunol* 2010; 162:12–23.
35. Pinter-Brown LC. SGN-30: A basis for the effective treatment of CD30 positive hematopoietic malignancies. *Expert Opin Investig Drugs* 2008; 17:1883–7.
36. Rickert RC, Jellusova J, Miletic AV. Signaling by the tumor necrosis factor receptor superfamily in B-cell biology and disease. *Immunol Rev* 2011; 244:115–33.
37. Gerber HP. Emerging immunotherapies targeting CD30 in Hodgkin's lymphoma. *Biochem Pharmacol* 2010; 79:1544–52.
38. van Kooten C, Banchereau J. CD40-CD40 ligand. *J Leukoc Biol* 2000; 67:2–17.
39. Tong AW, Stone MJ. Prospects for CD40-directed experimental therapy of human cancer. *Cancer Gene Ther* 2003; 10:1–13.
40. Treumann A, Lively MR, Schneider P, Ferguson MA. Primary structure of CD52. *J Biol Chem* 1995; 270:6088–99.
41. Gilleece MH, Dexter TM. Effect of Campath-1H antibody on human hematopoietic progenitors in vitro. *Blood* 1993; 82:807–12.
42. Boursalian TE, McEarchern JA, Law CL, Grewal IS. Targeting CD70 for human therapeutic use. *Adv Exp Med Biol* 2009; 647:108–19.
43. Borst J, Hendriks J, Xiao Y. CD27 and CD70 in T cell and B cell activation. *Curr Opin Immunol* 2005; 17:275–81.
44. Borghese F, Clanchy FI. CD74: An emerging opportunity as a therapeutic target in cancer and autoimmune disease. *Expert Opin Ther Targets* 2011; 15:237–51.
45. Stein R, Mattes MJ, Cardillo TM, Hansen HJ, Chang CH, Burton J, et al. CD74: A new candidate target for the immunotherapy of B-cell neoplasms. *Clin Cancer Res* 2007; 13:5556–63s.
46. Collins M, Ling V, Carreno BM. The B7 family of immune-regulatory ligands. *Genome Biol* 2005; 6:223.
47. Suvas S, Singh V, Sahdev S, Vohra H, Agrewala JN. Distinct role of CD80 and CD86 in the regulation of the activation of B cell and B cell lymphoma. *J Biol Chem* 2002; 277:7766–75.
48. Vyth-Dreese FA, Boot H, Dellemijn TA, Majoor DM, Oomen LC, Laman JD, et al. Localisation in situ of costimulatory molecules and cytokines in B-cell non-Hodgkin's lymphoma. *Immunology* 1998; 94:580–6.
49. Walsh SH, Rosenquist R. Immunoglobulin gene analysis of mature B-cell malignancies: Reconsideration of cellular origin and potential antigen involvement in pathogenesis. *Med Oncol* 2005; 22:327–41.
50. Jensen M, Tan G, Forman S, Wu AM, Raubitschek A. CD20 is a molecular target for scFvFc:zeta receptor redirected T cells: Implications for cellular immunotherapy of CD20+ malignancy. *Biol Blood Marrow Transplant* 1998; 4:75–83.
51. 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; 9:279–86.
52. Chen EY, Press OW, Jensen MC. CD8 immunomagnetic selection and interleukin-15 (IL-15) facilitate the isolation of human CD8 cytotoxic T-lymphocytes (CTL) genetically engineered to express an anti-CD20 chimeric T-cell receptor (cTCR). *J Clin Oncol* 2004; 22:2542.
53. Wang J, Press OW, Lindgren CG, Greenberg P, Riddell S, Qian X, et al. Cellular immunotherapy for follicular lymphoma using genetically modified CD20-specific CD8+ cytotoxic T lymphocytes. *Mol Ther* 2004; 9:577–86.
54. Kowolik CM, Topp MS, Gonzalez S, Pfeiffer T, Olivares S, Gonzalez N, et al. CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. *Cancer Res* 2006; 66:10995–11004.
55. Wang J, Jensen M, Lin Y, Sui X, Chen E, Lindgren CG, et al. Optimising adoptive polyclonal T cell immunotherapy of lymphomas, using a chimeric T cell receptor possessing CD28 and CD137 costimulatory domains. *Hum Gene Ther* 2007; 18:712–25.
56. 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; 17:1453–64.
57. James SE, Greenberg PD, Jensen MC, Lin Y, Wang J, Till BG, et al. Antigen sensitivity of CD22-specific chimeric TCR is modulated by target epitope distance from the cell membrane. *J Immunol* 2008; 180:7028–38.
58. Giordano Attianese GM, Marin V, Hoyos V, Savoldo B, Piszitola I, Tettamanti S, Agostoni V, et al. In vitro and in vivo model of a novel immunotherapy approach for chronic lymphocytic leukemia by anti-CD23 chimeric antigen receptor. *Blood* 2011; 117:4736–45.
59. Shaffer DR, Savoldo B, Yi Z, Chow KK, Kakarla S, Spencer DM, et al. T cells redirected against CD70 for the immunotherapy of CD70-positive malignancies. *Blood* 2011; 117:4304–14.
60. Vera J, Savoldo B, Vigouroux S, Biagi E, Pule M, Rossig C, et al. T lymphocytes redirected against the kappa light chain of human immunoglobulin efficiently kill mature B lymphocyte-derived malignant cells. *Blood*

- 2006; 108:3890–7.
61. Wang L, Abbasi F, Gaigalas AK, Vogt RF, Marti GE. Comparison of fluorescein and phycoerythrin conjugates for quantifying CD20 expression on normal and leukemic B-cells. *Cytometry B Clin Cytom* 2006; 70:410–15.
  62. Rossmann ED, Lundin J, Lenkei R, Mellstedt H, Osterborg A. Variability in B-cell antigen expression: Implications for the treatment of B-cell lymphomas and leukemias with monoclonal antibodies. *Hematol J* 2001; 2:300–6.
  63. Rocha B, Grandien A, Freitas AA. Anergy and exhaustion are independent mechanisms of peripheral T cell tolerance. *J Exp Med* 1995; 181:993–1003.
  64. Bataille R, Jégo G, Robillard N, Barillé-Nion S, Harousseau JL, Moreau P, et al. The phenotype of normal, reactive and malignant plasma cells. Identification of «many and multiple myelomas» and of new targets for myeloma therapy. *Haematologica* 2006; 91:1234–340.
  65. Moloney M, Shreffler WG. Basic science for the practicing physician: flow cytometry and cell sorting. *Ann Allergy Asthma Immunol* 2008; 101:544–9.
  66. Hombach AA, Schildgen V, Heuser C, Finnern R, Gilham DE, Abken H. T cell activation by antibody-like immunoreceptors: the position of the binding epitope within the target molecule determines the efficiency of activation of redirected T cells. *J Immunol* 2007; 178:4650–7.
  67. Lamers CH, Sleijfer S, Vulto AG, Kruit WH, Kliffen M, Debets R, et al. Treatment of metastatic renal cell carcinoma with autologous T-lymphocytes genetically retargeted against carbonic anhydrase IX: First clinical experience. *J Clin Oncol* 2006; 24:e20–2.
  68. Morgan RA, Yang JC, Kitano M, Dudley ME, Laurencot CM, Rosenberg SA. Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognising ERBB2. *Mol Ther* 2010; 18:843–51.
  69. 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* 2010; 18:666–8.
  70. Lamers CH, Willemsen R, van Elzaker P, van Steenbergen-Langeveld S, Broertjes M, Oosterwijk-Wakka J, et al. Immune responses to transgene and retroviral vector in patients treated with ex vivo-engineered T cells. *Blood* 2011; 117:72–82.
  71. Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, et al. Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biol Blood Marrow Transplant* 2010; 16:1245–56.
  72. van Lunzen J, Glausinger T, Stahmer I, von Baehr V, Baum C, Schilz A, et al. Transfer of autologous gene-modified T cells in HIV-infected patients with advanced immunodeficiency and drug-resistant virus. *Mol Ther* 2007; 15:1024–33.
  73. Newrzela S, Cornils K, Li Z, Baum C, Brugman MH, Hartmann M, et al. Resistance of mature T cells to oncogene transformation. *Blood* 2008; 112:2278–86.
  74. Hacein-Bey-Abina S, Hauer J, Lim A, Picard C, Wang GP, Berry CC, et al. Efficacy of gene therapy for X-linked severe combined immunodeficiency. *N Engl J Med* 2010; 363:355–64.
  75. Junghans, RP. Strategy escalation: An emerging paradigm for safe clinical development of T cell gene therapies. *J Transl Med* 2010; 8:55.