Published in final edited form as:
Best Pract Res Clin Haematol. 2008 September ; 21(3): 375–389. doi:10.1016/j.beha.2008.08.002.

Adoptive T-cell immunotherapy of chronic lymphocytic leukaemia

Aaron E. Foster, PhD [Assistant Professor], **Malcolm K. Brenner, MA, MD, PhD, FRCP, FRCPath [Professor]**, and **Gianpietro Dotti, MD [Associate Professor]***
Center for Cell and Gene Therapy, Baylor College of Medicine, The Methodist Hospital and Texas Children's Hospital, 6621 Fannin Street, MC 3-3320, Houston, Texas 77030, USA

Abstract

Immunotherapy for B-cell chronic lymphocytic leukaemia (B-CLL) and other haematological malignancies may consist of passive antibody, active immunization or adoptive T-cell transfer. This chapter will focus on T-lymphocyte immunotherapy; an approach supported by earlier observations that the beneficial effects of allogeneic stem cell transplantation depend, in part, on the graft-versus-leukaemia effects mediated by these cells. One promising strategy consists of the genetic manipulation of effector T lymphocytes to express tumour-specific T-cell receptors or chimeric antigen receptors directed against surface antigens on the B-CLL cells. This methodology is now being integrated with the concept that tumour recurrence may be due to the persistence of a reservoir of more primitive and chemoresistant tumour cells, dubbed ‘cancer stem cells’, with self-renewal capacity. Identification and characterization of these cancer stem cells in B-CLL is crucial for the development of new anti-tumour agents, and for the identification of target antigens for cellular immunotherapy. This chapter will describe how immunotherapy may be directed to a more primitive side population of B-CLL cells.

Keywords

chronic lymphocytic leukaemia; immunotherapy; adoptive T-cell transfer; chimeric antigen receptor; CD19; CD20; immunoglobulins; cancer stem cells

B-cell chronic lymphocytic leukaemia (B-CLL) is the most frequently diagnosed form of leukaemia in the Western world.[1] In more than 95% of patients, it is characterized by the clonal expansion of a small B-lymphocyte subset that co-expresses the CD5 surface marker distinct from most other peripheral blood B cells.[2] The clinical course of the disease is generally indolent, although several biological and clinical prognostic factors identify patients with more aggressive disease.[1,3,4] Early-stage B-CLL requires minimal intervention, but malignant lymphocytes accumulate progressively in lymph nodes, liver and spleen, and bone marrow failure may ultimately occur. Small molecule therapeutics such as fludarabine may diminish disease levels but overall survival is not prolonged significantly.[5] Similarly, passive immunotherapy with B-cell-specific monoclonal antibodies may modify immediate symptoms and signs, but does not lead to long-term disease-free survival.[6,7] More aggressive treatment with allogeneic stem cell transplantation (allo-SCT) may eradicate the disease[8], but even with subablative preparative regimens, transplant-related mortality remains significant, particularly in the older age group who are most commonly afflicted with the disease.[9]

The anti-leukaemia activity of allo-SCT is only partially a consequence of the intensive chemotherapy or radiotherapy used as a preparative regimen. In addition, the donor T-cell component of the graft likely contributes a significant graft-versus-leukaemia (GvL) effect.[9,

* Corresponding author. Tel.: +1 832 824 4725; Fax: +1 832 825 4732. gdotti@bcm.tmc.edu (G. Dotti).

[10] Unfortunately, this benefit is frequently associated with more generalized donor T-cell alloreactivity, causing graft-versus-host disease (GvHD) with considerable morbidity and mortality.[8] Nevertheless, the presence of the GvL effect in patients with B-CLL undergoing allo-SCT implies that these cells may be targeted effectively by effector T cells. Strategies that selectively amplify T cells that recognize tumour-specific antigens may produce therapeutic benefit without the adverse effects of more generalized alloreactivity.

Target Antigens for Adoptive T-Cell Immunotherapy of B-CLL

B-CLL cells may express or overexpress a number of tumour-associated antigens (TAAs) that can be the target of specific cytotoxic T-lymphocyte (CTL) responses.[11–13] These include fibromodulin, MDM2 (murine double minute 2), survivin, oncofetal antigen-immature laminin receptor protein (OFAiLRP), KW-2 and KW-13 (identified by serological screening of cDNA expression libraries or SEREX), preferentially expressed antigen of melanoma (PRAME) and receptor for hyaluronic-acid-mediated motility (RHAMM/CD168).[11] While these TAAs are expressed, often at high levels, by B-CLL cells, they are absent from most normal host tissues. B-CLL cells also express a unique monoclonal immunoglobulin, so the idiotypic determinants on this molecule may serve as true tumour-specific antigenic targets.[11]

$CD8^+$ and $CD4^+$ T lymphocytes that recognize TAAs can be identified and isolated from B-CLL patients and healthy donors.[12] However, TAAs are often poorly immunogenic and TAA-specific CTLs are rare and usually have low affinity for the antigen.[14] Moreover, tumour-specific CTLs in cancer patients may be anergic due to the inhibitory effects of the tumour micro-environment[15], or poorly functional as a consequence of extensive chemotherapy/radiation treatment. The generation of sufficient numbers of functionally potent TAA-specific CTLs for clinical trials remains challenging.

To overcome the limitation of isolating and expanding TAA-specific CTLs, it may be possible to combine this approach with active immunotherapy using gene-modified cancer vaccines.[16] For example, immunization prior to preparation of TAA-CTL should increase precursor frequency and simplify the process of CTL generation, while a vaccine boost following the adoptive transfer of the cells could further increase their in-vivo persistence and frequency. Vaccination with B-CLL tumour cells engineered to express CD40L certainly induces CLL-specific $CD4^+$ and $CD8^+$ T-cell immune responses[17,18], and if the logistical and regulatory impediments associated with such an approach can be overcome, this combination of active and passive immunotherapy may be of considerable value.

An alternative strategy is to use gene transfer to generate large numbers of T cells with defined anti-tumour specificity. Two technologies currently being studied for this purpose are the transfer of genes encoding a T-cell receptor (TCR) or a chimeric antigen receptor (CAR).

αβTCR Gene Transfer

The antigen specificity of T lymphocytes is determined by the expression of a ‘unique’ TCR, composed of α- and β-chains, which recognizes a specific protein-derived peptide presented in the context of major histocompatibilty complex (MHC) class I and class II molecules for $CD8^+$ cells and $CD4^+$ cells, respectively. Polyclonal T lymphocytes can be manipulated genetically to express transgenic TCR-α and TCR-β chains, specific for a peptide derived from a tumour antigen.[19] Moreover, transgenic T cells are biologically functional since they can specifically eliminate tumour cells expressing the tumour antigen recognized by the transgenic TCR.[19,20] Since B-CLL cells express several TAAs that are known to be processed and presented in the context of MHC molecules, TCR gene transfer may represent a valuable immunotherapy strategy.

The recent clinical translation of TCR gene transfer has been made possible by significant technical improvements in retroviral vector construction allowing for the co-expression of multiple genes, packaging cell lines, and protocols for optimizing primary T-cell transduction. Recently, feasibility of TCR gene transfer was demonstrated in a clinical study for melanoma patients.[19] Investigators at the National Cancer Institute demonstrated that adoptive transfer of polyclonal T cells transduced with a retroviral vector encoding the TCR-α and TCR-β chains of a MART1-specific TCR induced an objective clinical response in two of 15 melanoma patients.[19] In demonstrating the therapeutic potential of TCR gene transfer, the study offered potential strategies to optimize T-cell transduction. For example, TCR-α and TCR-β chains need to be coupled with CD3-associated molecules to be expressed on the cell surface, and free TCR-α and TCR-β chains are degraded rapidly. Transgenic TCRs have to compete with the native TCRs for CD3 binding, and the low availability of free CD3 molecules may significantly impair transgenic TCR receptor expression on the cell surface. Moreover, transgenic TCR-α and TCR-β chains may form heterodimers with native TCRs, not only further impairing the formation of the desired transgenic TCR, but also producing TCRs with unknown antigen specificity and potential autoreactivity.[20,21]

In the clinical trial described by Morgan et al, both the above concerns did not occur, since the transgenic MART-1-specific TCR was biologically functional and auto-immunity was not evident in the treated patients, albeit after relatively short follow-up.[19] However, these observations may not be applicable to other tumour models, and additional studies are in progress to identify anti-tumour TCR clones with high affinity/avidity for TAAs in which the transgenic TCR is modified to reduce the risk of cross-pairing and the formation of heterodimers (Figure 1a).[22,23]

Despite these encouraging results, two additional barriers limit the broad clinical application of this strategy. First, the specificity of the TCR is restricted by HLA molecules so that multiple receptors must be generated to cover common MHC polymorphisms. Although, in theory, a limited number of receptors may be sufficient to include more than 90% of the European Caucasian population[21], this would still require a massive effort of TCR cloning and validation in clinical trials. Secondly, tumour cells may escape T-cell recognition by downregulating MHC molecules or mutating/deleting the antigenic epitopes specifically recognized by the TCR.[24,25] The risk of selecting tumour deletion-mutants may be reduced by treating patients in a state of minimal residual disease after tumour debulking by conventional means, or by targeting antigens essential for maintaining the neoplastic phenotype. In addition, the combination of T-cell therapy with therapeutic agents that upregulate MHC expression in tumour cells may also reduce the risk of tumour escape.

Although the TCR gene transfer approach has not been tested in B-CLL, the promising results obtained in preclinical evaluation[26] suggest that this strategy could also be applied to antigens expressed in B-CLL such as PRAME and survivin.

Chimeric Antigen Receptor Gene Transfer

An alternative strategy for TCR transfer is the construction and transfer of genes encoding a chimeric antigen receptor into the T cells. CARs are artificial molecules usually composed of a specific-antigen-binding portion made of the variable domains of the heavy chain and light chain of a monoclonal antibody, linked together as a single-chain Fv antibody (scFv). To this is coupled a signalling region from either the ζ-chain of the TCR/CD3 complex or the γ-chain of the FcϵRI receptor (Figure 1b).[27,28] When expressed in T cells, CARs bind the antigen expressed on the cell surface of the target tumour cells through the scFv component, and trigger T-cell effector function through the FcR-γ or CD3-ζ domain.[27,28]

The major advantage of CAR gene transfer over TCR gene transfer is that recognition is MHC independent, since native molecules are recognized rather than processed proteins. Moreover, recognition can be extended to non-protein surface molecules, which are outside the scope of recognition of the conventional αβ-TCR.[29] Conversely, since most CARs are antibody derived, they can only recognize TAAs that are present on the cell surface, and unlike αβ-TCR, they cannot recognize 'internal' TAA targets that are nonetheless processed and presented by MHC molecules to the αβ-TCR.

B-CLL is particularly suited to attack by CAR expressing T cells, since there are several suitable TAAs that have been clinically validated, such as CD20[6], that may be targeted using specific monoclonal antibodies (Mabs). One might argue that since Mabs directed to these antigens fail to eliminate disease, T cells directed to the same target by a CAR will be similarly ineffectual. However, T cells exhibit multiple pathways for cell-mediated killing of tumour cells, can actively traffic to tumour sites, and can persist as memory cells.[30] As such, T cells targeted to TAAs such as CD20 may be more effective than Mabs in generating durable tumour responses, even when their specificity is the same as the Mabs.

CD20 and CD19 antigens appear in the early phase of B-cell development, and are almost invariably expressed at high levels on B-CLL cells.[1] T lymphocytes expressing transgenic CARs directed to CD19 or CD20 were highly effective at eliminating B-cell-derived malignancies in animal models[31–34], indicating that this strategy may have potential clinical benefits. CARs expressing T cells mediate their cytolytic effect through expression of perforin/granzyme-B and significant release of interferon-γ. One potential drawback of targeting CD20 or CD19 using CAR-modified T cells could be the extensive and prolonged elimination of the normal B-lymphocyte compartment and consequent impairment of humoral immunity. Although this side effect may often be clinically manageable by using human immunoglobulin infusion, a preferable target would be an antigen expressed more selectively by B-CLL cells, which would then allow preservation of humoral immunity. One possibility is to target the κ- or λ-light chain of human immunoglobulin, which is clonally expressed on B-CLL cells and other mature B-cell-derived malignancies. Hence, all κ+ (or λ+) B-CLL and normal B cells could be eliminated, whilst sparing the normal B-cell population expressing the reciprocal light chain (Figure 2).[35] Animal studies have shown that excellent disease protection is afforded provided that B cells expressing either κ or λ Ig are retained.[35,36] Finally, CD23, which is overexpressed in B-CLL cells and only weakly expressed on normal B cells, may be suitable as a target.[1]

Despite the clinical potential of CAR gene transfer, early clinical trials using CAR-redirected polyclonal T cells (including CAR-CD19-redirected cells) in patients with human immunodeficiency virus or cancer[37–40] did not show evidence of clinical response. In part, lack of clinical benefit may be attributed to the limited persistence of CAR-redirected T cells after adoptive transfer.[38,39] In addition, effective T-cell activation requires the presence of multiple co-stimulatory signals which undergo ligation in conjunction with the engagement of the antigen-specific receptor. In the absence of such co-stimulation, the response is aborted and T cells may become anergic. The majority of target tumour cells lack expression of ligands for the critical co-stimulatory molecule receptors on T cells, and since CAR molecules coupled only to the CD3-ζ domain can themselves provide just one signal to T lymphocytes following target antigen engagement, T-cell activation is incomplete. Hence, 'second-generation CARs' have been constructed in which the CD3-ζ domain has been combined in cis with specific endodomains derived from different co-stimulatory molecules including CD28, ICOS, CD134 or CD137 (Figure 1b).[23,35,41–43] When T cells expressing these new constructs encounter tumour-target antigens with their CAR, they receive both CD3-ζ and co-stimulatory domain signals, and produce cytokines such as interleukin (IL)-2 that enhance their expansion, function and persistence. Like other tumours, B-CLL cells lack expression of most co-stimulatory

molecules, and clinical studies testing CD19- and CD20-specific CARs coupled to the CD28 endodomain are now in progress.

T-Cell Manipulation in Patients with B-CLL

Genetic modification of T cells from B-CLL patients may be technically problematic. B-CLL cells secrete a high level of immunomodulatory factors, including tumour growth factor-β and IL-10, that generate an immunosuppressive tumour environment.[15] As a consequence, T lymphocytes in patient peripheral blood are often functionally defective and contain a high proportion of cells with regulatory phenotype ($CD4^+/CD25^+/FoxP3^+$) and function.[18] These Treg can also impair the function of genetically modified T cells.[44] Fortunately, second-generation CARs, which include the CD28 co-stimulatory endodomain, are resistant to the inhibitory effects of Treg cells.[44] The molecular mechanisms underlying this resistance still need to be elucidated. T lymphocytes isolated from the peripheral blood of B-CLL patients can be manipulated genetically to express CAR molecules (CAR-CD19-CD28 or CAR-κ-light chain-CD28) and to acquire cytotoxic activity against autologous tumour cells (Figure 2).[35] Clinical trials will determine whether these cells remain functionally active after adoptive transfer even in an immunosuppressive tumour environment of B-CLL patients. In these trials, B-CLL patients will be infused with autologous T cells genetically modified to express CAR-CD19 or CAR-κ-light chain. Dose escalation will be used in a phase I study, in which patients will receive $2 \times 10^7/m^2$, $1 \times 10^8/m^2$ or $2 \times 10^8/m^2$ cells in one single dose, and safety, persistence of adoptively transferred T cells, and effects on the leukaemic and normal B lymphocytes will be evaluated. If detrimental effects from the B-CLL cells are discovered, adoptive transfer of CAR-redirected T cells could be combined with conventional treatment to minimize tumour burden or Treg numbers[45], and the CAR T cells themselves may be modified to counteract tumour inhibitory factors such as tumour growth factor-β.[46]

Cancer Stem/Progenitor Cells and B-CLL

Almost all malignancies contain cells that are heterogeneous in phenotype and proliferative potential. Recently, the concept of ‘cancer stem cells’ (or progenitor or tumour-initiating/repopulating cells) has been elevated in its importance because of their apparent role in tumourigenesis, drug resistance and relapse. In haematological malignancies, there is compelling evidence for the presence of cancer stem cells, since differences in proliferative potential between defined subsets can be readily observed in vitro using colony forming assays[47], and in vivo by engraftment studies in immuno-deficient mice (NOD/SCID).[48] These and other studies show that only a fraction of tumour cells are able to reinitiate tumour colonies.[49] As tumour subpopulations based on phenotypic markers (e.g. $CD34^+CD28^-$ in acute myeloid leukaemia) or functional characteristics [e.g. Hoechst 33342 side population (SP) and aldehyde dehydrogenase activity] can reconstitute a heterogeneous cancer, many tumours may possess self-renewal and differentiation programmes that are similar to normal adult stem cells.[49] The distinction between cancer stem cells (self-renew and differentiate) and cancer progenitor cells (differentiate but do not self-renew) is often not made clear in studies of this putative phenomenon. However, for the purposes of targeted therapy, the term can be defined operationally: a cancer stem cell is a cancer cell whose eradication leads to the subsequent and progressive regression of the remaining non-stem-cell tumour.

Is there a B-CLL Proliferative Compartment?

B-CLL has long been considered a disease of ‘accumulation’ of anergic B cells which are defective for apoptosis or programmed cell death.[50,51] B-CLL tumour cells overexpress several anti-apoptosis genes, including Bcl-2[52,53] and Mcl-1[54], supporting the notion that this form of chronic leukaemia is the result of the abnormal lifespan of malignant B cells. In addition, B-

CLL tumour cells are hyporesponsive to polyclonal B-cell stimuli compared with normal B cells[55], setting the B-CLL disease course apart from the uncontrolled proliferation which characterizes many forms of acute leukaemia.

Recently, there has been a change in perspective in which both accumulation (resistance to death) and proliferation (growth) contribute to the pathobiology of B-CLL.[56] This shift has been driven by the importance of 'proliferative centres' in the lymphoid organs of B-CLL patients, and the identification of new cell survival and activation signals in these malignant B cells. B-CLL tumour cells in the peripheral blood rarely progress through the cell cycle.[57,58] In contrast, focal aggregates of proliferating tumour cells (so-called 'pseudo-follicles') can be found in the lymph nodes and bone marrow of many B-CLL patients. Granziero et al demonstrated that these pseudo-follicles contained B-CLL tumour cells expressing the cell cycle marker Ki-67 and survivin, a member of the inhibitor of apoptosis family.[13] Proliferation of B-CLL in lymph nodes may be mediated by environmental stimuli, including ligation of CD40 on B-CLL cells by CD40L (CD154) on activated $CD4^+$T lymphocytes.[13] Studies that measure birth and death rates of B-CLL by labelling DNA of dividing cells[56,59,60] using deuterium (^2H) have shown that B-CLL cells are newly generated at rates similar to or even greater than normal B cells.[60] However, not all B-CLL tumour cells proliferate at equal rates. $CD5^+CD19^+CD38^+$ cells incorporate higher levels of ^2H than $CD38^-$ cells, and in addition, the $CD38^+$ subset co-express markers of activation and proliferation including CD69, CD62L, ZAP-70 and Ki-67.[61] Given that peripheral blood B-CLL expresses high levels of p27 (also called 'cyclin-dependent kinase 1 B' or 'Kip1')[62], and that lymph nodes and bone marrow appear to contain proliferating tumour cells, it seems likely that the cells found in the lymphoid compartment contribute to the accumulation of B-CLL observed in the peripheral blood, and that targeting this small fraction of proliferating tumour cells may produce longer-term remission.[56,63]

Is there a B-CLL Stem Cell Compartment?

B-CLL manifests a heterogenic phenotype in which cells exhibit diverse levels of responsiveness to chemotherapy. To date, however, identification of the subpopulation responsible for drug resistance and relapse has proven elusive. One means of linking stem cell properties with cytotoxic drug resistance is the identification of an SP of cells on fluorescence flow cytometry by means of their capacity to efflux vital dyes.[64] Over the past several years, a primitive SP cell subset with marrow repopulating ability has been defined in normal bone marrow from many mammals, including humans, using the fluorescent dyes Rhodamine 123 and Hoechst 33342.[64,65] For example, SP cells obtained from normal marrow have a $CD34^{low/neg}$ phenotype and can repopulate lethally irradiated mice in serial transplantation models.[64] The association between the SP phenotype and progenitor/stem cell enrichment has been supported in a variety of tissues in addition to bone marrow, including skeletal muscle, liver, brain, mammary, kidney, skin, testis and retinal tissue.[66] Due to this wide biological distribution of SP cells, the present authors and others reasoned that malignancies might also harbour SP cells with tumour stem cell properties. The intrinsic dye efflux capacity of these cells should be associated with rapid expulsion of many cytotoxic drugs, perhaps allowing the cells to survive treatment and produce relapse.

The present authors initially reported a malignant $CD45^+CD34^{low/neg}$ SP cell subset in more than 80% of patients with acute myeloid leukaemia.[67] These cells generated $CD45^+CD34^+$ malignant haematopoietic progenitor cells functioning as leukaemic stem cells. As the cells also expelled lipophilic antileukaemic drugs, the data suggested that the SP subset is capable of contributing to relapse.[67] The authors also found malignant SP cells in solid tumours, such as neuroblastoma[68], in which 15 of 23 primary neuroblastomas contained $CD45^-CD71^-GD2^+$ SP cells. Although the authors could not precisely assign a position to SP

cells in the developmental hierarchy of neuroblastoma malignancy, the phenotypic characteristics of the SP subset, such as high expression of GD2 and stem cell factor receptor, together with their greater replicative potential in cell culture and high expression of two transporter proteins, ABCA3 and ABCG2, are all consistent with proximity to an early cell in the hierarchy of neural crest progenitor development. Other groups have now reported SP cells in additional solid tumours, including gliomas[69], hepatocellular carcinoma[70] and breast cancer.[71,72] Importantly, these studies indicate that the SP subset is enriched for tumourigenic cells (i.e. cells that have the capacity to form new tumours in vitro and in vivo), supporting the hypothesis that cancer stem cells/progenitor cells play an important role in tumourigenesis.

As anticipated from the above results, SP cells are readily found in many other haematological malignancies, including multiple myeloma[73] and adult T-cell leukaemia/lymphoma.[74] The SP phenotype is again associated with resistance to anti-neoplastic agents.[73] The present authors have since examined B-CLL primary tumour samples for the presence of SP cells, and found 0.1–2% SP cells amongst the circulating $CD5^+CD19^+$ B-CLL cells in 12 of 15 (80%) patients studied (Figure 3), suggesting that in this disease there is a phenotypically defined subpopulation that may contribute to disease resistance. Overall, the evidence that SP cells exist in both haematological and non-haematological tumours, and are enriched for tumour-initiating cells and resistant to chemotherapy, indicates that targeting this population in B-CLL may be critical in order to eradicate the disease.

Targeted Immunotherapy for B-CLL ‘Stem Cell’ Compartments

As described above, effector T cells can, in principle, be used to eradicate malignancies[30,75–78], but this approach can only work if the T cells target antigens present on any putative stem cell subset. B-CLL cells express many TAAs, including RHAMM/CD168, fibromodulin, PRAME and PANE1.[79,80] Since cancer stem cells differ phenotypically from bulk tumour cells, it is critical that the target antigens are expressed on cancer stem cells, and desirable that they should be critical to the maintenance of the malignancy. Targeting the antigens expressed on both leukaemia stem cells and ‘mature’ leukaemic blasts has been shown to be feasible using CTLs specific for minor histocompatibility antigens (mHags).[81,82] In acute myeloid leukaemia, for example, mHag-specific CTLs recognize the stem cell compartment ($CD34^+CD38^-$) and prevent engraftment and disease.[48,82] Antigens that are overexpressed in drug-resistant (SP) cancer stem cells can also be exploited as CTL targets. Polycomb group genes such as Bmi-1 and EZH2 are recognized by tumour infiltrating lymphocytes and in-vitro-generated CTLs.[83] Bmi-1 contributes to self-renewal and regulating proliferative activity in normal stem and progenitor cells[84,85], while EZH2 is necessary for cellular proliferation[86], so target antigens may well meet the dual criteria of being present in the malignant stem cell and essential for its survival and expansion. There are concerns, however, that these antigens are also expressed in the normal stem cell compartment and that targeting them may produce severe toxicity.

Since B-CLL also contains a phenotypically distinct putative stem cell population, this too may be targeted by immune mechanisms. The authors are currently identifying and validating potential target antigens for CTLs. Of note, it may be possible to use the bulk B-CLL population as a ‘surrogate’ stimulator to generate a specific anti-SP cell response. One of the observations made in vaccine studies using CD40-activated B-CLL cells as the immunogen is that the activated B-CLL cells not only upregulate the co-stimulatory molecules CD80 and CD86[12,16,87–89] and dramatically increase the ability of the B-CLL cells to recruit T-cell responses[13], but also increase their expression of potential target antigens such as survivin and p53.[90] These antigens are also highly expressed in B-CLL SP cells, but not in the unstimulated bulk B-CLL population. Hence a combination of adoptive immunotherapy with T cells and

booster vaccinations with CD40-activated B-CLL cells may ensure effective and sustained targeting of antigens expressed in the putative stem cells of B-CLL.

Conclusions

The only known curative treatment for B-CLL is allogeneic stem cell transplantation; an effect produced, at least in part, by the GvL activity of donor T cells. To avoid concomitant GvHD, investigators have prepared T cells directed to TAAs that are more restricted in their tissue distribution. The clinical use of transgenic T cells expressing B-CLL-directed receptors is in its infancy, but as knowledge of the molecular markers of B-CLL improves and understanding of the cellular biology of the immune system increases, this approach is likely to become progressively more feasible and effective. It will, however, be important to ensure that these B-CLL-specific T cells are directed towards antigens that are present in any stem or precursor cell malignant population, and are not limited to the cells of mature tumours.

Acknowledgments

This work was supported, in part, by the Leukemia and Lymphoma Society Specialized Center of Research (Grant No. 7018) and the NCI Specialized Programs of Research Excellence (Grant P50CA126752). GD is also supported by the Doris Duke Charitable Foundation/Clinical Scientist Development Award and by a Leukemia and Lymphoma Society Translational Research grant.

References

1. Rozman C, Montserrat E. Chronic lymphocytic leukemia. The New England Journal of Medicine 1995;333:1052–1057. [PubMed: 7675049]
2. Fialkow PJ, Najfeld V, Reddy AL, et al. Chronic lymphocytic leukaemia: clonal origin in a committed B-lymphocyte progenitor. Lancet 1978;2:444–446. [PubMed: 79806]
3. Lee JS, Dixon DO, Kantarjian HM, et al. Prognosis of chronic lymphocytic leukemia: a multivariate regression analysis of 325 untreated patients. Blood 1987;69:929–936. [PubMed: 3814821]
4. Montserrat E, Gomis F, Vallespi T, et al. Presenting features and prognosis of chronic lymphocytic leukemia in younger adults. Blood 1991;78:1545–1551. [PubMed: 1884021]
5. Rai KR, Peterson BL, Appelbaum FR, et al. Fludarabine compared with chlorambucil as primary therapy for chronic lymphocytic leukemia. New England Journal of Medicine 2000;343:1750–1757. [PubMed: 11114313]
6. O'Brien SM, Kantarjian H, Thomas DA, et al. Rituximab dose-escalation trial in chronic lymphocytic leukemia. Journal of Clinical Oncology 2001;19:2165–2170. [PubMed: 11304768]
7. Osterborg A, Dyer MJ, Bunjes D, et al. Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. European Study Group of CAMPATH-1H Treatment in Chronic Lymphocytic Leukemia. Journal of Clinical Oncology 1997;15:1567–1574. [PubMed: 9193354]
8. Pavletic ZS, Arrowsmith ER, Bierman PJ, et al. Outcome of allogeneic stem cell transplantation for B cell chronic lymphocytic leukemia. Bone Marrow Transplantation 2000;25:717–722. [PubMed: 10745256]
9. Schetelig J, Thiede C, Bornhauser M, et al. Evidence of a graft-versus-leukemia effect in chronic lymphocytic leukemia after reduced-intensity conditioning and allogeneic stem-cell transplantation: the Cooperative German Transplant Study Group. Journal of Clinical Oncology 2003;21:2747–2753. [PubMed: 12860954]
10. Rondon G, Giralt S, Huh Y, et al. Graft-versus-leukemia effect after allogeneic bone marrow transplantation for chronic lymphocytic leukemia. Bone Marrow Transplantation 1996;18:669–672. [PubMed: 8879640]
11. Giannopoulos K, Schmitt M. Targets and strategies for T-cell based vaccines in patients with B-cell chronic lymphocytic leukemia. Leukemia & Lymphoma 2006;47:2028–2036. [PubMed: 17071473]

12. Biagi E, Dotti G, Yvon E, et al. Molecular transfer of CD40 and OX40 ligands to leukemic human B cells induces expansion of autologous tumor-reactive cytotoxic T lymphocytes. Blood 2005;105:2436–2442. [PubMed: 15536147]

13. Granziero L, Ghia P, Circosta P, et al. Survivin is expressed on CD40 stimulation and interfaces proliferation and apoptosis in B-cell chronic lymphocytic leukemia. Blood 2001;97:2777–2783. [PubMed: 11313271]

14. Johnson LA, Heemskerk B, Powell DJ Jr, et al. Gene transfer of tumor-reactive TCR confers both high avidity and tumor reactivity to nonreactive peripheral blood mononuclear cells and tumor-infiltrating lymphocytes. Journal of Immunology 2006;177:6548–6559.

15. Gorgun G, Holderried TA, Zahrieh D, et al. Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. The Journal of Clinical Investigation 2005;115:1797–1805. [PubMed: 15965501]

16. Kater AP, van Oers MH, Kipps TJ. Cellular immune therapy for chronic lymphocytic leukemia. Blood 2007;110:2811–2818. [PubMed: 17638850]

*17. Wierda WG, Cantwell MJ, Woods SJ, et al. CD40-ligand (CD154) gene therapy for chronic lymphocytic leukemia. Blood 2000;96:2917–2924. [PubMed: 11049967]

*18. Biagi E, Rousseau R, Yvon E, et al. Responses to human CD40 ligand/human interleukin-2 autologous cell vaccine in patients with B-cell chronic lymphocytic leukemia. Clinical Cancer Research 2005;11:6916–6923. [PubMed: 16203783]

*19. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science 2006;314:126–129. [PubMed: 16946036]

20. Schumacher TN. T-cell-receptor gene therapy. Nature Reviews. Immunology 2002;2:512–519.

21. Stauss HJ, Cesco-Gaspere M, Thomas S, et al. Monoclonal T-cell receptors: new reagents for cancer therapy. Molecular Therapy : the Journal of the American Society of Gene Therapy 2007;15:1744–1750. [PubMed: 17637721]

22. Kuball J, Dossett ML, Wolfl M, et al. Facilitating matched pairing and expression of TCR chains introduced into human T cells. Blood 2007;109:2331–2338. [PubMed: 17082316]

23. Maher J, Brentjens RJ, Gunset G, et al. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRzeta/CD28 receptor. Nature Biotechnology 2002;20:70–75.

24. Khong HT, Restifo NP. Natural selection of tumor variants in the generation of ‘tumor escape’ phenotypes. Nature Immunology 2002;3:999–1005. [PubMed: 12407407]

25. Gottschalk S, Ng CY, Perez M, et al. An Epstein-Barr virus deletion mutant associated with fatal lymphoproliferative disease unresponsive to therapy with virus-specific CTLs. Blood 2001;97:835–843. [PubMed: 11159505]

26. Xue SA, Gao L, Hart D, et al. Elimination of human leukemia cells in NOD/SCID mice by WT1-TCR gene-transduced human T cells. Blood 2005;106:3062–3067. [PubMed: 16020516]

27. Hedrick SM. Chimeric T cell receptor-immunoglobulin molecules: function and applications. International Reviews of Immunology 1993;10:279–290. [PubMed: 8360589]

*28. Eshhar Z, Waks T, Gross G, et al. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. Proceedings of the National Academy of Sciences of the United States of America 1993;90:720–724. [PubMed: 8421711]

29. Rossig C, Bollard CM, Nuchtern JG, et al. Targeting of G(D2)-positive tumor cells by human T lymphocytes engineered to express chimeric T-cell receptor genes. International Journal of Cancer 2001;94:228–236.

30. Heslop HE, Ng CY, Li C, et al. Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes. Nature Medicine 1996;2:551–555.

31. Jensen MC, Cooper LJ, Wu AM, et al. Engineered CD20-specific primary human cytotoxic T lymphocytes for targeting B-cell malignancy. Cytotherapy 2003;5:131–138. [PubMed: 12745575]

*32. Cooper LJ, Topp MS, Serrano LM, et al. T-cell clones can be rendered specific for CD19: toward the selective augmentation of the graft-versus-B-lineage leukemia effect. Blood 2003;101:1637–1644. [PubMed: 12393484]

33. Rossig C, Brenner MK. Chimeric T-cell receptors for the targeting of cancer cells. Acta Haematologica 2003;110:154–159. [PubMed: 14583676]

34. Brentjens RJ, Latouche JB, Santos E, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nature Medicine 2003;9:279–286.

*35. Vera J, Savoldo B, Vigouroux S, et al. T-lymphocytes redirected against the kappa light chain of human immunoglobulin efficiently kill mature B-lymphocyte derived malignant cells. Blood 2006;108:3890–3897. [PubMed: 16926291]

36. Zegers BJ, Maertzdorf WJ, Van Loghem E, et al. Kappa-chain deficiency. An immunoglobulin disorder. New England Journal of Medicine 1976;294:1026–1030. [PubMed: 815819]

37. Mitsuyasu RT, Anton PA, Deeks SG, et al. Prolonged survival and tissue trafficking following adoptive transfer of CD4zeta gene-modified autologous CD4(+) and CD8(+) T cells in human immunodeficiency virus-infected subjects. Blood 2000;96:785–793. [PubMed: 10910888]

38. Kershaw MH, Westwood JA, Parker LL, et al. A phase I study on adoptive immunotherapy using gene-modified T cells for ovarian cancer. Clinical Cancer Research 2006;12:6106–6115. [PubMed: 17062687]

39. Park JR, Digiusto DL, Slovak M, et al. Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma. Molecular Therapy : the Journal of the American Society of Gene Therapy 2007;15:825–833. [PubMed: 17299405]

40. Jensen MC, Popplewell L, DiGiusto, et al. A first-in-human clinical trial of adoptive therapy using CD19-specific chimeric antigen receptor re-directed T-cells for recurrent/refractory follicular lymphoma [abstract]. Blood 2008;110:92a.

*41. Finney HM, Lawson AD, Bebbington CR, et al. Chimeric receptors providing both primary and costimulatory signaling in T cells from a single gene product. Journal of Immunology 1998;161:2791–2797.

42. Pule MA, Straathof KC, Dotti G, et al. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. Molecular Therapy : the Journal of the American Society of Gene Therapy 2005;12:933–941. [PubMed: 15979412]

43. Kowolik CM, Topp MS, Gonzalez S, 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 Research 2006;66:10995–11004. [PubMed: 17108138]

44. Loskog A, Giandomenico V, Rossig C, et al. Addition of the CD28 signaling domain to chimeric T-cell receptors enhances chimeric T-cell resistance to T regulatory cells. Leukemia 2006;20:1819–1828. [PubMed: 16932339]

45. Dannull J, Su Z, Rizzieri D, et al. Enhancement of vaccine-mediated antitumor immunity in cancer patients after depletion of regulatory T cells. The Journal of Clinical Investigation 2005;115:3623–3633. [PubMed: 16308572]

46. Bollard CM, Rossig C, Calonge MJ, et al. Adapting a transforming growth factor beta-related tumor protection strategy to enhance antitumor immunity. Blood 2002;99:3179–3187. [PubMed: 11964281]

47. Park CH, Bergsagel DE, McCulloch EA. Mouse myeloma tumor stem cells: a primary cell culture assay. Journal of the National Cancer Institute 1971;46:411–422. [PubMed: 5115909]

*48. Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nature Medicine 1997;3:730–737.

49. Reya T, Morrison SJ, Clarke MF, et al. Stem cells, cancer, and cancer stem cells. Nature 2001;414:105–111. [PubMed: 11689955]

50. Dameshek W. Chronic lymphocytic leukemia – an accumulative disease of immunologically incompetent lymphocytes. Blood 1967;29(Suppl):84.

51. Caligaris-Cappio F, Hamblin TJ. B-cell chronic lymphocytic leukemia: a bird of a different feather. Journal of Clinical Oncology 1999;17:399–408. [PubMed: 10458259]

52. Pezzella F, Tse AG, Cordell JL, et al. Expression of the bcl-2 oncogene protein is not specific for the 14;18 chromosomal translocation. The American Journal of Pathology 1990;137:225–232. [PubMed: 2201196]

53. Schena M, Larsson LG, Gottardi D, et al. Growth- and differentiation-associated expression of bcl-2 in B-chronic lymphocytic leukemia cells. Blood 1992;79:2981–2989. [PubMed: 1375120]
54. Kitada S, Zapata JM, Andreeff M, et al. Bryostatin and CD40-ligand enhance apoptosis resistance and induce expression of cell survival genes in B-cell chronic lymphocytic leukaemia. British Journal of Haematology 1999;106:995–1004. [PubMed: 10520003]
55. Lankester AC, van Schijndel GM, van der Schoot CE, et al. Antigen receptor nonresponsiveness in chronic lymphocytic leukemia B cells. Blood 1995;86:1090–1097. [PubMed: 7620163]
56. Chiorazzi N, Ferrarini M. Evolving view of the in-vivo kinetics of chronic lymphocytic leukemia B cells. Hematology / the Education Program of the American Society of Hematology 2006;512:273–278.
57. Andreeff M, Darzynkiewicz Z, Sharpless TK, et al. Discrimination of human leukemia subtypes by flow cytometric analysis of cellular DNA and RNA. Blood 1980;55:282–293. [PubMed: 6928106]
58. Meinhardt G, Wendtner CM, Hallek M. Molecular pathogenesis of chronic lymphocytic leukemia: factors and signaling pathways regulating cell growth and survival. Journal of Molecular Medicine 1999;77:282–293. [PubMed: 10023781]
59. Hellerstein MK. Measurement of T-cell kinetics: recent methodologic advances. Immunology Today 1999;20:438–441. [PubMed: 10500289]
60. Messmer BT, Messmer D, Allen SL, et al. In vivo measurements document the dynamic cellular kinetics of chronic lymphocytic leukemia B cells. The Journal of Clinical Investigation 2005;115:755–764. [PubMed: 15711642]
61. Damle RN, Temburni S, Calissano C, et al. CD38 expression labels an activated subset within chronic lymphocytic leukemia clones enriched in proliferating B cells. Blood 2007;110:3352–3359. [PubMed: 17684154]
62. Vrhovac R, Delmer A, Tang R, et al. Prognostic significance of the cell cycle inhibitor p27Kip1 in chronic B-cell lymphocytic leukemia. Blood 1998;91:4694–4700. [PubMed: 9616167]
63. Ringshausen I, Peschel C, Decker T. Mammalian target of rapamycin (mTOR) inhibition in chronic lymphocytic B-cell leukemia: a new therapeutic option. Leukemia & Lymphoma 2005;46:11–19. [PubMed: 15621776]
64. Goodell MA, Brose K, Paradis G, et al. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. The Journal of Experimental Medicine 1996;183:1797–1806. [PubMed: 8666936]
*65. Goodell MA, Rosenzweig M, Kim H, et al. Dye efflux studies suggest that hematopoietic stem cells expressing low or undetectable levels of CD34 antigen exist in multiple species. Nature Medicine 1997;3:1337–1345.
66. Challen GA, Little MH. A side order of stem cells: the SP phenotype. Stem Cells 2006;24:3–12. [PubMed: 16449630]
67. Wulf GG, Wang RY, Kuehnle I, et al. A leukemic stem cell with intrinsic drug efflux capacity in acute myeloid leukemia. Blood 2001;98:1166–1173. [PubMed: 11493466]
68. Hirschmann-Jax C, Foster AE, Wulf GG, et al. A distinct ‘side population’ of cells with high drug efflux capacity in human tumor cells. Proceedings of the National Academy of Sciences of the United States of America 2004;101:14228–14233. [PubMed: 15381773]
69. Kondo T, Setoguchi T, Taga T. Persistence of a small subpopulation of cancer stem-like cells in the C6 glioma cell line. Proceedings of the National Academy of Sciences of the United States of America 2004;101:781–786. [PubMed: 14711994]
70. Chiba T, Kita K, Zheng YW, et al. Side population purified from hepatocellular carcinoma cells harbors cancer stem cell-like properties. Hepatology 2006;44:240–251. [PubMed: 16799977]
71. Patrawala L, Calhoun T, Schneider-Broussard R, et al. Side population is enriched in tumorigenic, stemlike cancer cells, whereas ABCG2+ and ABCG2- cancer cells are similarly tumorigenic. Cancer Research 2005;65:6207–6219. [PubMed: 16024622]
72. Zhou J, Wulfkuhle J, Zhang H, et al. Activation of the PTEN/mTOR/STAT3 pathway in breast cancer stem-like cells is required for viability and maintenance. Proceedings of the National Academy of Sciences of the United States of America 2007;104:16158–16163. [PubMed: 17911267]
73. Matsui W, Wang Q, Barber JP, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. Cancer Research 2008;68:190–197. [PubMed: 18172311]

74. Kayo H, Yamazaki H, Nishida H, et al. Stem cell properties and the side population cells as a target for interferon-alpha in adult T-cell leukemia/lymphoma. Biochemical and Biophysical Research Communications 2007;364:808–814. [PubMed: 17977513]
75. Rooney CM, Smith CA, Ng CY, et al. Use of gene-modified virus-specific T lymphocytes to control Epstein-Barr-virus-related lymphoproliferation. Lancet 1995;345:9–13. [PubMed: 7799740]
76. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with antitumor lymphocytes. Science 2002;298:850–854. [PubMed: 12242449]
77. Straathof KC, Bollard CM, Popat U, et al. Treatment of nasopharyngeal carcinoma with Epstein-Barr virus-specific T lymphocytes. Blood 2005;105:1898–1904. [PubMed: 15542583]
78. Bollard CM, Gottschalk S, Leen AM, et al. Complete responses of relapsed lymphoma following genetic modification of tumor-antigen presenting cells and T-lymphocyte transfer. Blood 2007;110:2838–2845. [PubMed: 17609424]
79. Giannopoulos K, Li L, Bojarska-Junak A, et al. Expression of RHAMM/CD168 and other tumor-associated antigens in patients with B-cell chronic lymphocytic leukemia. International Journal of Oncology 2006;29:95–103. [PubMed: 16773189]
80. Brickner AG, Evans AM, Mito JK, et al. The PANE1 gene encodes a novel human minor histocompatibility antigen that is selectively expressed in B-lymphoid cells and B-CLL. Blood 2006;107:3779–3786. [PubMed: 16391015]
*81. Bonnet D, Warren EH, Greenberg PD, et al. CD8(+) minor histocompatibility antigen-specific cytotoxic T lymphocyte clones eliminate human acute myeloid leukemia stem cells. Proceedings of the National Academy of Sciences of the United States of America 1999;96:8639–8644. [PubMed: 10411928]
82. Rosinski KV, Fujii N, Mito JK, et al. DDX3Y encodes a class I MHC-restricted H-Y antigen that is expressed in leukemic stem cells. Blood 2008;111:4817–4826. [PubMed: 18299450]
83. Steele JC, Torr EE, Noakes KL, et al. The polycomb group proteins, BMI-1 and EZH2, are tumour-associated antigens. British Journal of Cancer 2006;95:1202–1211. [PubMed: 17024127]
84. Raaphorst FM, Meijer CJ, Fieret E, et al. Poorly differentiated breast carcinoma is associated with increased expression of the human polycomb group EZH2 gene. Neoplasia 2003;5:481–488. [PubMed: 14965441]
85. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature 2003;423:255–260. [PubMed: 12714970]
86. Bracken AP, Pasini D, Capra M, et al. EZH2 is downstream of the pRB-E2F pathway, essential for proliferation and amplified in cancer. The EMBO Journal 2003;22:5323–5335. [PubMed: 14532106]
87. Ranheim EA, Kipps TJ. Activated T cells induce expression of B7/BB1 on normal or leukemic B cells through a CD40-dependent signal. The Journal of Experimental Medicine 1993;177:925–935. [PubMed: 7681471]
88. Takahashi S, Rousseau RF, Yotnda P, et al. Autologous antileukemic immune response induced by chronic lymphocytic leukemia B cells expressing the CD40 ligand and interleukin 2 transgenes. Human Gene Therapy 2001;12:659–670. [PubMed: 11426465]
89. Wierda WG, Kipps TJ, Keating MJ. Novel immune-based treatment strategies for chronic lymphocytic leukemia. Journal of Clinical Oncology 2005;23:6325–6332. [PubMed: 16155015]
90. Laytragoon-Lewin N, Rossmann ED, Castro J, et al. Significance of phosphotyrosine proteins, Bcl-2 and p53 for apoptosis in resting B-chronic lymphocytic leukemia (CLL) cells. International Journal of Cancer 2002;97:344–348.

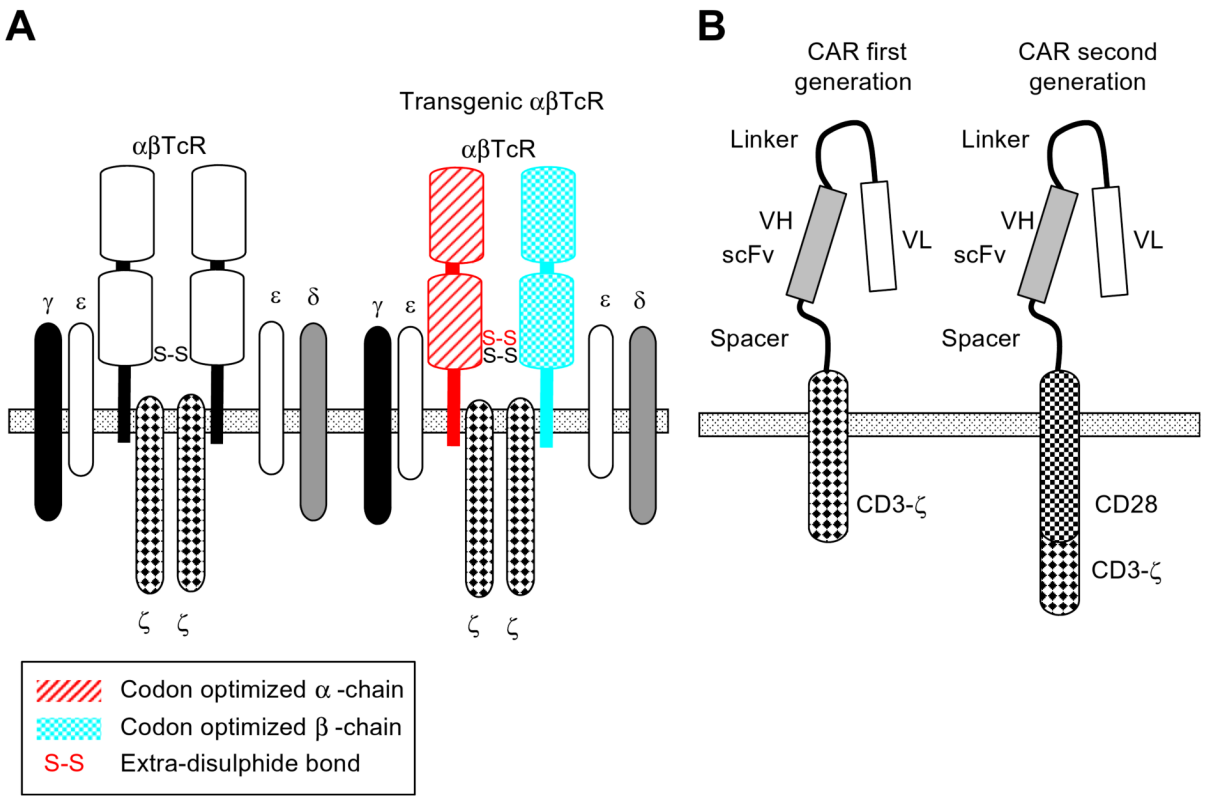

Figure 1.

Structure of transgenic T-cell receptors (TCRs) and chimeric antigen receptors (CARs). (A) T lymphocytes expressing the native TCR/CD3 complex and the transgenic TCR. Two strategies to maximize the expression of the transgenic TCR are also illustrated: in codon optimization of the sequences of TCR-α and TCR-β; and the inclusion of a second disulphide bond corresponding to the constant region of the TCR. (B) Schematic structure of first- and second-generation CARs. Second-generation CARs contain one or more co-stimulatory endodomains (illustrated with CD28), cloned in frame with the scFv and the CD3-ζ endodomain.

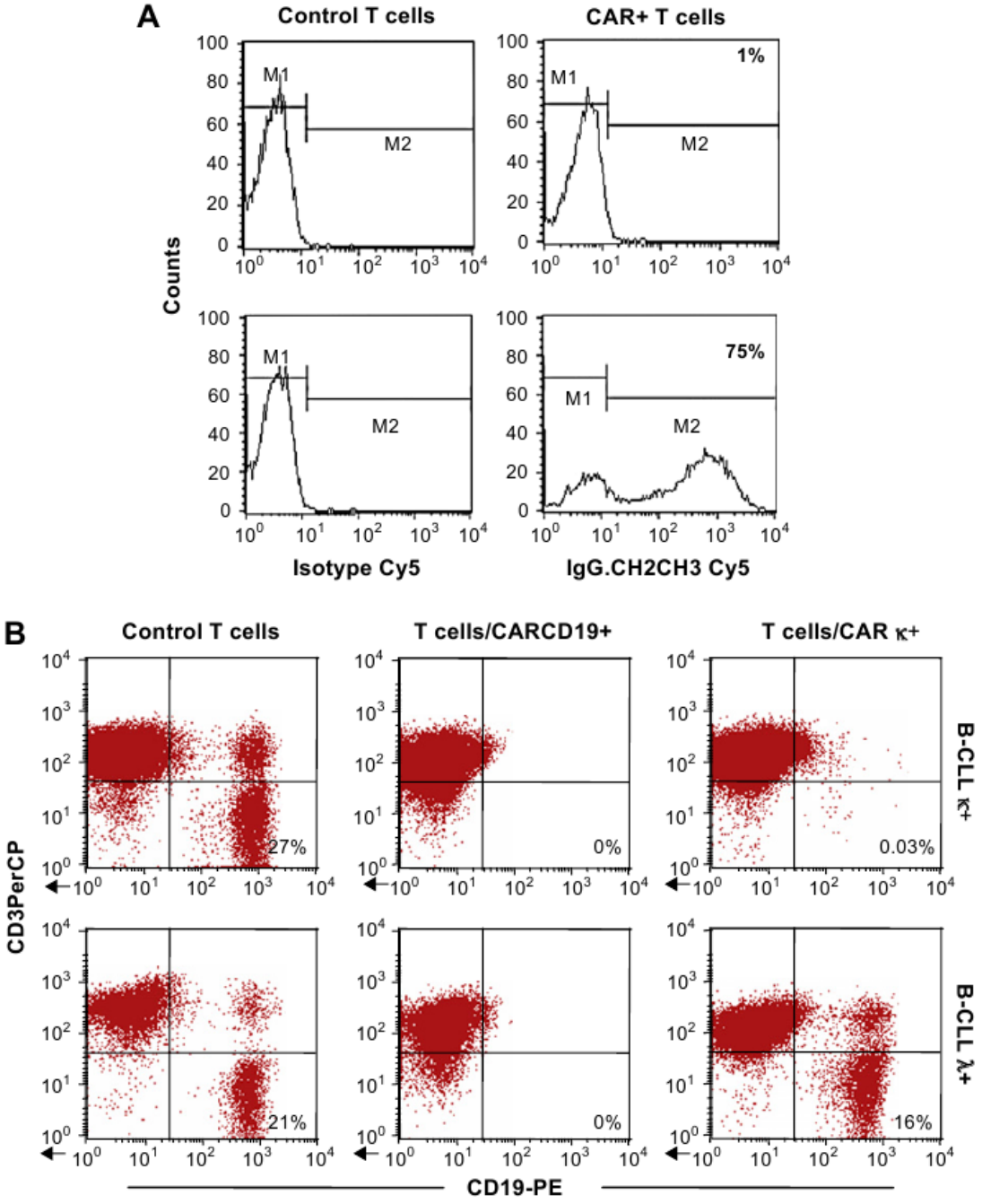

Figure 2.

Chimeric antigen receptor (CAR)-redirected T lymphocytes can eliminate B-cell chronic lymphocytic leukaemia (B-CLL) cells. T-lymphocytes isolated from the peripheral blood of B-CLL patients were transduced either with control-GFP vector, or CAR CD19-CD28ζ or CAR κ-light-chain-CD28ζ vectors. (A) Expression of the CARs on transgenic T cells detected by flow cytometry using an antibody recognizing the spacer region of the CARs. (B) After transduction, T cells were co-cultured with autologous B-CLL cells expressing either κ^+ or λ^+ (ratio 5:1). After 3–4 days of culture, cells were collected and stained with CD3-PerCP and CD19-PE and analysed by flow cytometry to evaluate the growth of $CD19^+$ B-CLL cells. No $CD19^+/\kappa^+$ cells were detectable after co-culture with CAR CD19-CD28ζ or CAR κ-light-chain-

CD28ζ T cells, while $CD19^{+}/\lambda^{+}$ cells were detectable when tumour cells were co-cultured with control T cells or CAR κ-light-chain-CD28ζ T cells. This experiment illustrates that T cells redirected to target the κ-light chain expressed on the cell surface of B-CLL can eliminate tumour cells as efficiently as CAR CD19-redirected T cells, but also spare B cells expressing the λ-light chain.

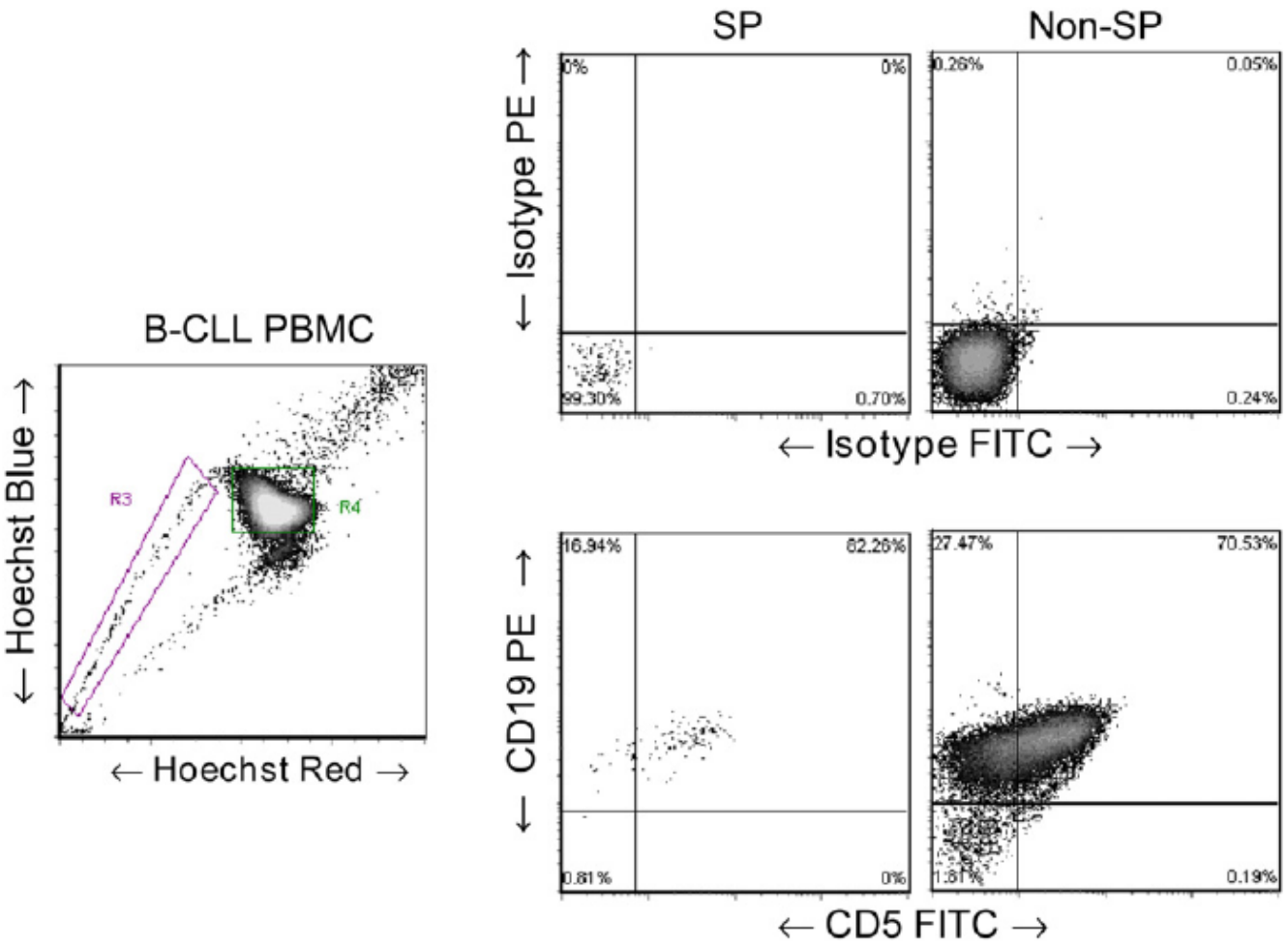

Figure 3.

B-cell chronic lymphocytic leukaemia (B-CLL) primary tumour cells contain a distinct side population (SP) phenotype. Primary leukaemic cells isolated from the peripheral blood of B-CLL patients were stained with Hoechst 33342 for 90 mins, and then labelled with either isotype control antibodies or CD5 FITC and CD19 PE to distinguish B-CLL cells from normal B cells. SP cells are $CD5^{+}CD19^{+}$ and are not present in the peripheral blood of healthy donors (not shown).