Over the past decade substantial advances have been made in understanding the biological and molecular mechanisms of chronic lymphocytic leukaemia (CLL). The evaluation of new chemotherapeutic combinations has led to an increase in the rate of complete remission in patients with CLL. In addition, the use of monoclonal antibodies such as rituximab and alemtuzumab has added substantial benefit when combined with chemotherapy. However, the ability to eradicate disease at a molecular level, and improve clinically relevant patient outcome measures, continue to pose difficult challenges. Regardless of the stage of the disease, most patients will relapse after initial treatment and become refractory to salvage chemotherapy with median overall survival ranging from 10 to 19 months. Thus, the need to develop alternative therapies to kill leukaemic cells, or to fight relapse, remains a hot topic under intense investigation. Targeting cell-surface molecules present on leukaemic B-cells with T-cells transfected with chimeric antigen receptors (CAR) may be an attractive immunotherapeutic strategy to reduce the leukaemic cell burden.

CAR can be engineered by combining an antigen-specific monoclonal antibody using its variable chain fragments with a T-cell activating signalling receptor in a single fusion protein. Once this modified protein is expressed on the surface of a T-cell, and binds to its specific antigen, an activation signal is transmitted into the T-cell. This latter will trigger its effector functions to lyse the target cell. Typically, T-cells expressing CAR react like conventional T-cells, but attach to the target antigen by the variable chain fragments of the monoclonal antibody, and so are named T-bodies. Since its first description, CAR design has evolved over the years with the goal of enhancing T-cell signalling functions [Figure 1]. The first generation of CAR consisted of heavy and light chain immunoglobulin variable regions fused in a single chain and coupled to signalling modules, which are normally present in the T-cell receptor complex such as the CD3zeta-chain. This first generation of CAR effectively redirected T-cell cytotoxicity, but failed to enable T-cell proliferation and survival upon repeated antigen exposure, and anti-tumour responses were limited. The second generation of CAR incorporated another signalling receptor from co-stimulatory molecules such as CD28, CD134 or CD137 to reduce activation-induced cell death and improve T-cell survival. The third generation of CAR incorporated two co-stimulatory molecules: CD28, CD134 or CD137 in a sequence fused to CD3-zeta chain and were designed to further enhance killing functions, proliferation capacities and production of survival cytokines such as interleukin-2. Compared to classical T-cell-based immunotherapies, T-cells expressing-CAR present several attractive advantages including obviating the need for recognising peptide presentation by major histocompatibility complex, the ability to target a range of tumour surface antigens, and relatively

A New Cellular Weapon to Kill Leukaemic B-Cells

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rapid generation within one to four weeks.\textsuperscript{5,7,8} Although the clinical value of genetically engineered T-cells is still to be validated, recent data from two studies reported that CAR targeting CD19 (CART19) was able to kill leukaemic B-cells expressing this surface antigen and that tumour control was sustained for 10 months following this therapy.\textsuperscript{9,10} The CART19 was designed to express a single chain variable fragment derived from an anti-CD19 specific antibody along with a CD137 signalling domain and the CD3\textsubscript{zeta}-chain. T-cells expressing CART19 were generated by transfecting autologous T-cells from each CLL patient with a lentiviral vector, which express the CART19 construct. Prior to receiving a low dose of CART19, patients received lymphodepleting chemotherapy with pentostatin and cyclophosphamide and, 4 days later, $1.42 \times 10^7$ of engineered CART19 cells were administrated without additional cytokines or monoclonal antibodies. Two to three weeks after CART19 immunotherapy, patients developed a tumour lysis syndrome, which correlated positively with an increase in the number of circulating T-cells expressing CART19. Three to four days later the tumour lysis syndrome subsided without evidence of disease on physical examination. There was no palpable adenopathy and no evidence of CLL in the bone marrow. In addition, computed tomography (CT) scans showed a resolution of adenopathies. Six to 10 months following CART19 infusion, two of three subjects showed a complete remission with no residual CLL found by means of physical examination, CT scans, flow-cytometry and cytogenetic analyses. Normal B cells however continued to be lacking. Of note, each infused CART19 cell eradicated on average about 1,000 malignant cells. T-cell expressing CART19 underwent robust expansion, persisted at high levels in both circulating blood and bone marrow for at least 6 months and, most importantly, a proportion of these T-cells expressed memory markers and retained anti-CD19 effector functions. As expected the most frequent side effects, in addition to the tumour lysis syndrome, were B-cell lymphopenia and hypogammaglobulinaemia but these undesirable conditions should be manageable in CLL patients.

Overall, these small pilot, prospective, single-centre studies yielded very encouraging results as two of the three patients enrolled in the clinical trial had p53 gene deletion, which predicts poor survival, non-response to therapy and rapid progression. It also provides a proof of principle that CAR represents a promising approach to treat CLL patients and possibly other B-cell malignancies.\textsuperscript{11,12} Indeed, this therapy resulted in partial remission.
in a patient with follicular lymphoma for up to 32 weeks.11 In a more recent study, three patients with bulky CLL and one patient with B-cell acute lymphoblastic exhibited a response to CART19 containing CD28 as a co-stimulatory molecule.12

Accordingly, CART19 is generating substantial enthusiasm and its clinical value will probably be evaluated in large clinical trials. However, one potential concern is that co-stimulatory signals may lead to uncontrolled CAR T-cell proliferation thereby increasing the long term risk of toxicity by depleting non-tumour cells, which are important for homeostatic functions. It remains to be seen whether the long term side effects of CART19 will be acceptable or not. Another major safety issue is the theoretical risk of inducing oncogenic mutations after DNA integration of the vector. Previous reports have shown the occurrence of T-cell acute lymphoblastic leukaemia in four children treated with gene-transfer stem cells to correct their X-linked severe combined immunodeficiency.13 However, in contrast to haematopoietic stem cells, retroviral vector integration to mature T-cells has been found to be a safe strategy as demonstrated by long-term engraftment of donor lymphocytes genetically engineered with the suicide gene thymidine kinase of herpes simplex virus after allogeneic stem cell transplantation.14,15 Treatment with CART19 is an innovative immunotherapeutic approach to target leukaemic B-cells in patients with advanced chemotherapy-resistant CLL.

Certainly, this approach is still in its infancy for clinical use, but it constitutes a new weapon against B-cell neoplasms and potentially a model to further improve the curative potential of cellular therapies in patients for whom conventional therapies have failed.

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