ELSEVIER

Seminars in Oncology

Vaccine- and Immune-Based Therapy in Chronic Lymphocytic Leukemia

Rifca Le Dieu and John Gribben

B-cell chronic lymphocytic leukemia (CLL) would appear to be an ideal target of T-cell–mediated responses against the cancer cell. The cancer arises in cells that can act as antigen-presenting cells (APCs), CLL cells express tumor antigens, and the cells can be a target of the allogeneic T cells in a graft-versus-leukemia effect. Despite these potential benefits, immune responses against CLL cells have been difficult to elicit. CLL induces immune defects in the host, the tumor cells are inefficient APCs, and therapies given to patients with CLL are themselves immunosuppressive. Successful vaccination approaches in this disease will require steps to overcome these difficulties, including steps to improve the immune defects in this disease, identification of the targets of the immune response to monitor immmunologic responses, and improved presentation of antigen.
Semin Oncol 33:220-229

Paul Ehrlich first postulated in 1909 that the incidence of cancer would be much greater were it not for immune surveillance. However, the existence of such an immune response was doubted for much of the 20th century due to concerns as to the applicability to humans of information derived from studies of transplantable murine tumors. An article from the *British Journal of Cancer* in 1976 reported no evidence of an immune response to 27 different spontaneous tumors and concluded that: "transplanted tumor systems. . .entail artifactual immunity associated with viral or chemical induction."[1] It was not until a report in 1985 that it became apparent that the progression of human cancers could be reversed by immunologic manipulation when interleukin-2 (IL-2) was administered to humans with metastatic renal cell carcinoma and melanoma.[2] Since that time much effort has been spent on trying to gain an understanding of the way in which the immune system interacts with malignant cells and to manipulate that interaction for therapeutic benefit. Potential reasons why patients might fail to mount an effective T-cell–mediated immune response against their cancer are shown in Fig 1. The requirements for such an immune response are the presence of tumor-associated antigen in the cancer cell that can be effectively processed and presented by major histocompatibility complex (MHC) molecules. Also needed are functionally active T cells that are capable of recognizing this appropriately presented antigen. If all these steps occur, T-cell recognition should lead to tumor regression. Cancers of the immune system are logical places to begin to examine these questions for if the body cannot mount an immune response against a tumor derived from cells of the immune system, it is much less likely to have an effect against an epithelial tumor. Chronic lymphocytic leukemia (CLL) represents an attractive model in which to study such immunotherapeutic approaches. It is a slow-growing malignancy, which allows time for an immune response against the tumor cells to be generated. It is easy to obtain tumor cells as they circulate in large numbers in the peripheral blood. Furthermore, B-CLL cells express MHC class I and II in addition to a specific tumor antigen—the idiotype (Id)—a tumor-specific epitope of the immunoglobulin (Ig) B-cell receptor. CLL is characterized by immune deficiency and both humoral and T-cell functional defects have been documented. Although the B-CLL cells express tumor antigen there is no effective immune response against them despite evidence of leukemia-reactive T cells.[3] The malignant B cells fail to act as effective antigen-presenting cells (APC) or activators of costimulatory pathways. Baseline expression of MHC class I is normal but this molecule fails to undergo upregulation in response to interferon gamma (IFNγ).[4] In addition, the cells are resistant to FAS (CD95) ligand-mediated apoptosis.[5] Although the T cells are not part of the malignant clone, defects also occur in this population. There is an inversion of the CD4/CD8 ratio[6] with an increase in absolute numbers of phenotypically activated CD4 and CD8 cells.[7] CD4 cells are more susceptible to FAS ligand-induced apoptosis.[8] An oligoclonal TCR Vβ gene pattern is expressed[9–11] and there is an altered production of cytokines in-

Institute of Cancer, CRUK Medical Oncology Unit, Barts and The London School of Medicine, Queen Mary, University of London, London, UK.
Address correspondence to John Gribben, MD, DSc, Institute of Cancer, CRUK Medical Oncology Unit, Barts and The London School of Medicine, Queen Mary, University of London, Charterhouse Square, London EC1M 6BQ, UK. E-mail: john.gribben@cancer.ork.uk

 0093-7754/06/$-see front matter
doi:10.1053/j.seminoncol.2005.12.012

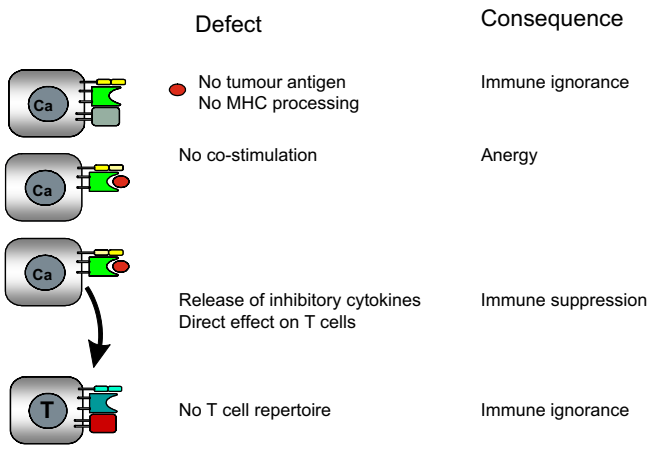

Figure 1 Mechanisms of failure to mount an immune response against cancer.

cluding interleukin-4 (IL-4) and IFNγ and also reduced expression of CD154 (CD40 ligand [CD40L]), the zeta chain of the TCR, and CD28.[12,13] Gene expression analysis has revealed differentially expressed genes mainly involved in cell differentiation in CD4 cells and defects in cytoskeletal formation, vesicle trafficking, and cytotoxicity in CD8 cells of CLL patients.[14] These changes can be induced in T cells from healthy allogeneic donors in coculture experiments with CLL B cells (Fig 2). Direct cellular contact is required for these changes in gene expression to occur and they appear not to be cytokine-mediated. These defects may explain the reduced mitogenic and allogeneic T-cell responses observed in the disease.[15] Inhibition of antitumor responses has been linked to the presence of inhibitory factors in patients. These may include $CD4^+$ $CD25^{high}$ regulatory T cells and patients with CLL have a significantly increased frequency and suppressive function of regulatory T cells, an abnormality that is corrected after therapy with fludarabine.[16]

Correcting the Immune Defects in CLL

In 1978, a case report was published of a 76-year-old woman with CLL and hepatosplenomegaly. An attempt was made to make her B-CLL cells more immunogenic by undertaking extracorporeal irradiation of the blood. This resulted in involution of the spleen and no macroscopic evidence of CLL could be found at subsequent autopsy.[17] Further attempts were made to improve the antigenicity of CLL cells by altering membrane cholesterol levels using cholesterylhemisuccinate with some clinical response.[18] More recently attention has focused on specific CLL cell surface molecules, particularly CD40, a member of the tumor necrosis factor (TNF) receptor family that is expressed throughout B-cell development and is implicated in cell survival and differentiation. Activation of CD40 improves the antigen-presenting capacity of B-CLL cells resulting in upregulation of costimulatory molecules (CD80, CD86) and subsequent T-cell proliferation (Fig 3).[19–21] Activation with CD40L can generate T-cell lines that proliferate specifically in response to unstimulated CLL

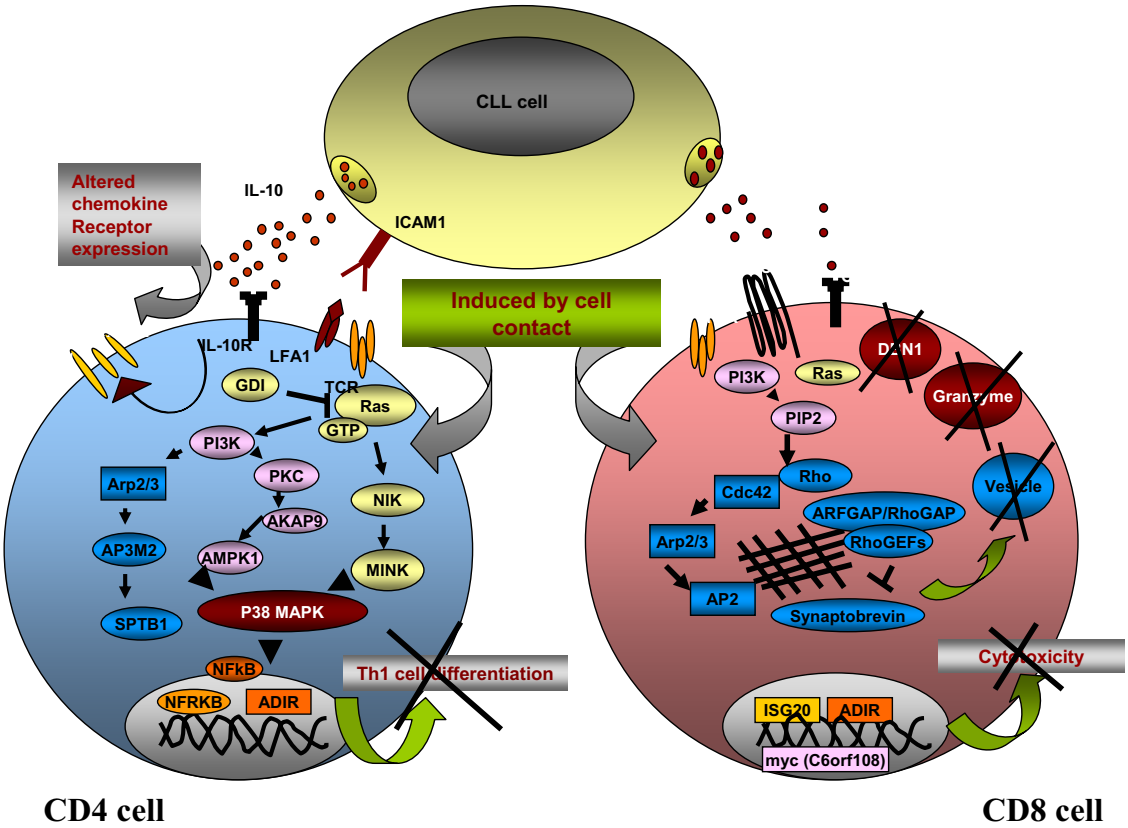

Figure 2 Interactions of CLL cells with T cells induces specific T-cell defects.

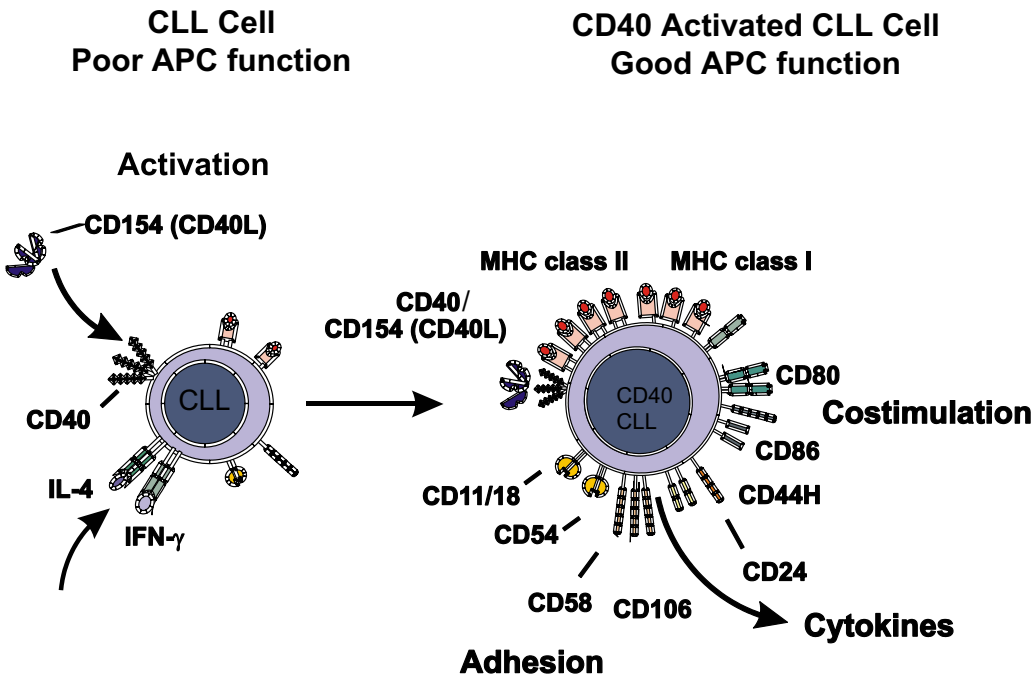

Figure 3 CD40 activation of CLL cells induces good antigen-presenting cell function.

cells. A cytotoxic response against CLL cells is seen using allogeneic healthy donor T cells, whereas autologous donor T cells will only lyse allogeneic CLL cells. This may be due to expansion of different effector populations,[22–24] or potentially due to the immune defects that occur in the tumor bearing host including the induction of tumor antigen specific anergy. Perversely, CD40 activation enhances the anti-apoptotic profile of CLL cells and increases the APC activity,[25,26] suggesting that there is a balance between survival of these cells or their elimination by an effective immune response.

T-cell unresponsiveness has been targeted directly in several ways. T cells have been genetically engineered to target the CD19 antigen aberrantly expressed on CLL B cells. These show ability to effectively lyse tumor cells in CLL and non-Hodgkin's lymphoma (NHL).[27,28] Mixed lymphocyte reactions with third-party stimulators (allogeneic Epstein-Barr virus [EBV]-transformed B-cell line with a human leukocyte antigen [HLA] different to that of the host) generate CD8 T cells with marked cytolytic activity against human CLL cells in a human-mouse radiation chimera.[29] These may have a potential role in stem cell transplantation. Incubating peripheral blood mononuclear cells (PBMC) with anti-CD3 and anti-CD28 monoclonal antibodies conjugated to superparamagnetic beads corrects T-cell defects dramatically in vitro and has been the subject of early clinical trials.[30]

Vaccine Development

A primary requirement for vaccine development is the identification of antigens expressed on tumor cells. Proteins that are either mutated or selectively or abundantly expressed in malignant but not in normal cells are potentially tumor-associated antigens (TAA). The ideal tumor antigen would be expressed exclusively in tumor tissue and be detectable in the majority of patients. Most tumor antigens do not possess these ideal characteristics and are therefore limited in their use. The risk of using an antigen with broader expression in healthy tissues is the induction of autoimmune phenomena.

Idiotype

B-cell malignancies have an obvious tumor antigen in the form of the idiotype. In fact, idiotype was the first tumor antigen described.[31–33] Each normal B cell expresses an immunoglobulin with unique variable region sequences in the heavy and light chains that together form the antigen-binding site. When a B cell undergoes malignant transformation, this idiotype is maintained by the malignant clone and can thus be regarded as a TAA. Idiotype peptides contain structures that can be recognized by antibodies and by CD4 and CD8 T cells. Mouse lymphoma models have shown that humoral and cellular mechanisms against idiotypes are effective in inducing tumor regression.[34] Clinical trials using vaccination have shown efficacy with mainly humoral responses.[35,36] Of note, all these trials have been in patients with B-cell lymphomas with no results published in patients with CLL. Although the use of tumor-derived idiotype protein to elicit an anti-tumor response is an attractive idea, the broader use of idiotypic vaccines has been hampered by the fact that not only is autologous Id weakly immunogenic but it is patient-specific so that the vaccine must be individually prepared for each patient. At least for CD8 T cells it appears that a small set of shared Ig-derived peptides could be presented on MHC class I molecules on malignant B cells that are recognized by CD8 T cells, potentially reducing the need for patient-specific vac-

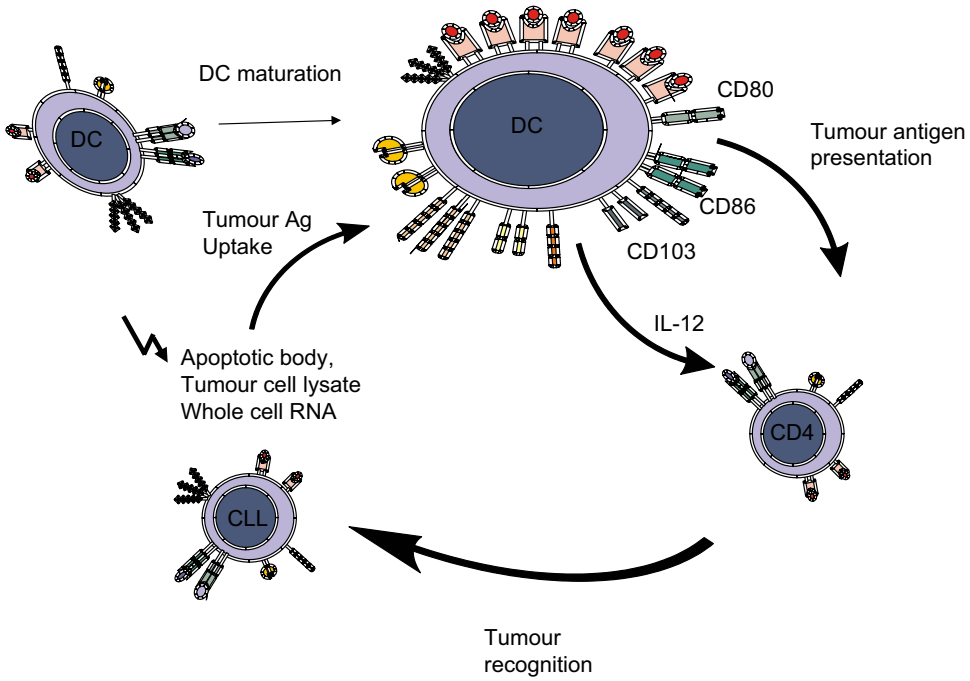

Figure 4 CLL antigen presentation by dendritic cells.

cines.[37,38] These epitopes are only weakly immunogenic but can be improved by altering the MHC binding residues (heteroclitic peptides). Attempts have been made to improve the immunogenicity of idiotype vaccines by coupling the peptide to an immunogenic carrier protein such as keyhole limpet hemocyanin (Id-KLH) 34 or granulocyte-macrophage colony-stimulating factor (GM-CSF).[39,40]

Other CLL Tumor Antigens

Several alternative TAA have been identified in CLL, including JD118 antigen,[41] onco-fetal antigen immature laminin receptor protein (OFA-iLRP),[42] survivin,[43] and SLLP1,[44] which all represent possible targets for immunotherapy. A series of fourteen potential tumor antigens in CLL were identified by serologic SEREX, whereby peptides within recombinantly expressed phage libraries were recognized by autologous and allogeneic sera from CLL patients. Three were novel antigens. Twelve peptides were investigated for their potential to induce cytotoxic T-cell responses. Specific cytotoxic T lymphocytes (CTLs) could be generated but these were unfortunately unable to lyse native CLL cells, raising the possibility that these antigens may instead represent T-helper cell antigens.[45] Additional approaches that are being used to identify tumor antigens include subtractive hybridization and bioinformatics approaches, attempting to identify putative antigens with limited expression in CLL.

Altered Tumor as an Antigen Source

In view of the difficulty in finding appropriate tumor antigens in CLL, alternative approaches have been taken. One such method involves presenting the whole CLL cell by professional APCs. One such approach involved subjecting patients' blood to a combination of oxidative physicochemical stressors in a blood treatment unit. This approach is based on release of antigen-binding heat-shock proteins and free radicals that increase the immunogenicity of CLL cells in vivo without requiring precise identification of antigenic targets. These oxidised autologous tumor cells are then re-injected into the patient and CLL-reactive T cells subsequently analyzed. 25 patients at various stages of disease progression were included in the study and it was found that CLL-reactive T-cell frequencies increased in response to vaccination in those patients who had pre-existing CLL-reactive T cells and that there was an inverse correlation between disease stage and anti-CLL T-cell reactivity.[3]

This approach has also been used in a phase I/II trial during the "watch and wait" phase of CLL. Eighteen patients at risk of disease progression and with white blood cell counts between 15 and 100 $\times$ 10^6 cells/mL were vaccinated using three different dosing schedules. Partial clinical responses associated with enhanced anti-tumor T-cell activity in vitro were observed in five patients.[46]

Dendritic Cells

One means of improving immune response to antigen is to use a more powerful antigen presenting cell—dendritic cells (DCs).[47–49] DC vaccines have been shown to have clinical activity in melanoma,[50,51] prostate carcinoma,[52,53] renal cell carcinoma,[54] and carcinoembryonic antigen–expressing cancers.[55] They have also been used with some success to generate an immune response against idiotype peptides in patients with follicular lymphoma. Of 35 patients vaccinated, 65% mounted T-cell or humoral anti-Id responses and of the 18 patients with residual tumor at the time of vaccination and four had tumor regression. Seventy percent remained without tumor progression at a median of 43 months after che-

motherapy. Six patients with disease progression after primary vaccine received booster vaccines and tumor regression was observed in three.[56]

A different approach is to use DCs pulsed with tumor lysate or apoptotic bodies as a means of effectively presenting unknown tumor antigens to the patient immune system (Fig 4). Pre-clinical studies have used autologous DCs pulsed with tumor cell lysate to generate CLL-specific immune responses in vitro.[23] CLL T cells stimulated with CLL-lysate pulsed DCs showed a significant increase in IFNγ secretion and specific cytotoxicity to autologous B cell targets but none to autologous T- or B-cell targets from healthy volunteers.[57] Both HLA class I and II CLL-specific CTL responses were induced using DCs transfected by electroporation with total tumor RNA or polymerase chain reaction (PCR)-amplified RNA derived from CLL cells.[58] Another method of improving DC-induced T-cell responses to CLL is to pretreat the T cells with interleukin 15 prior to culturing with lysate-pulsed autologous DCs.[59] Using DCs electrofused with CLL B cells (fusion hybrids) produced higher levels of specific cytotoxicity to CLL cells than using DCs alone, although another group came to the converse conclusion when comparing fusion hybrids with DCs pulsed with B-CLL apoptotic bodies.[60] A clinical trial involving nine patients with early stage CLL has been reported.[61] Monocyte-derived DCs obtained from unrelated healthy donors were stimulated with patient tumor cells as lysate or apoptotic bodies then injected intradermally once every 2 to 3 weeks. There were no signs of autoimmunity and only mild skin reactions. A decrease in peripheral blood leucocytes and CD19/CD5 leukemic cells was observed.

Trioma Cells

An alternative form of antigen presentation makes use of trioma cells. These are malignant B cells that have been fused to an anti-FcR hybridoma. The trioma cells express tumor-derived antigens and have anti-APC specificity. The trioma cell then binds to the FcR of an APC resulting in uptake and presentation of antigens. This method has been used in mice against B cell lymphoma and has induced long-lasting tumor immunity, with both CD4 and CD8 responses.[62] In a preclinical study, malignant cells from 11 patients with CLL were fused to anti-Fc receptor hybridomas. Trioma cells were successfully generated in seven cases and CLL-specific T-cell responses could be generated in vitro.[63]

Gene Therapy

As described above, an effective method to increase the APC capacity of CLL cells is through CD40 activation. One such approach involves introduction of CD154 into the CLL cells. Several strategies have been now been developed to accomplish this using adenovirus, recombinant adeno-associated virus, and herpes simplex virus vectors, as well as molecular transfer from fibroblasts that overexpress the ligand.[64–67] This CD154 gene therapy has been tested in a phase I clinical trial in CLL patients infusing adenoviral CD154 transfected CLL cells and resulted in increased or de novo expression of costimulatory molecules on bystander non-infected CLL cells. Patients developed high plasma levels of IL-2 and IFNγ, as well as increased numbers of leukemia-specific T cells as assessed by ELISPOT and mixed lymphocyte reactions.[68] Use of certain cytokines such as IL-2 improves the ability of CLL cells to express costimulatory molecules and to subsequently stimulate T-cell proliferation.[69] When CLL cells are cotransfected with CD154 and IL-2, they induce T-cell activation and autologous T cells capable of specifically recognizing parental CLL cells.[64]

Monoclonal Antibody Therapy

Despite the intense research efforts described above, active immunotherapy has had only limited success. Passive immunotherapy however has had more encouraging results. The concept dates back to Paul Ehrlich's hypothesis of the "magic bullet" that described what is now known as a monoclonal antibody binding to a cell surface receptor resulting in targeted killing of tumor cells with minimal damage to normal tissues.[70] To date, monoclonal antibody therapy is the only immune based therapy that has entered standard clinical care and two antibodies, rituximab and alemtuzumab, have been widely used in CLL patients.

Rituximab is a chimeric anti-CD20 monoclonal antibody that has been rapidly adopted by physicians for a wide range of B-cell malignancies because of its activity, its lack of cross-resistance with chemotherapy, and its minimal side effects. Disappointing results in small lymphocytic leukemia (SLL)/CLL in the pivotal trial[71] led to early skeptism but subsequent studies using alternative schedules, doses,[72,73] and patient populations[74] have clearly demonstrated efficacy. Rituximab alone produces predominantly partial responses.

Alemtuzumab is a human IgG monoclonal antibody directed against the CD52 antigen expressed on normal and leukemic B and T lymphocytes, macrophages, and monocytes. It therefore induces a profound lymphopenia, with subsequent risk of infectious complications. In the pivotal trial the response rate was 33%, including complete responses (CRs) in 2% of 93 patients with refractory disease treated at 21 centers.[75] Unlike rituximab, alemtuzumab has its most pronounced effects on the peripheral blood and bone marrow with minimal effect on bulky disease. Of note it is also effective in patients with the cytogenetic abnormality del 17p (loss of p53), which is associated with poor response to purine analogues and rituximab.[76]

Particular attention is now being paid to whether there are potential mechanisms of synergy using monoclonal antibodies in combination with chemotherapy to improve response. The Cancer and Leukemia Group B (CALGB) conducted a randomized phase II trial of concurrent versus sequential fludarabine and rituximab in previously untreated patients. The overall response rate in the concurrent arm was 90% including CRs in 47%.[77] The combination of fludarabine, rituximab, and cyclophosphamide (FCR) has also been investigated as first-line therapy in a single-center study with good preliminary results (71% CR rate with 57% of complete responders showing molecular remission) and also has sig-

Table 1 Reported Clinical Trials of Reduced Intensity Conditioning Allogeneic Stem Cell Transplant in CLL

Trial	No. of Patients	Disease Status at Transplant	Conditioning Regimen	Stem Cell Source	Median Follow-up	GVHD	Outcome
Khouri et al, 1998[96]	15 (6 with CLL)	6 refractory relapse, 2 induction failures	Fludarabine 90–150 mg/m^2 and cyclophosphamide 900–2,000 mg/m^2	PBSCs from HLA-identical siblings	180 d	5 aGVHD 1-3 1 fatal grade 4 aGVHD 2 cGVHD (1 fatal)	11 engrafted 8 CR
Schetelig et al, 2003[97]	30	All advanced CLL	Fludarabine/busulfan/ antithymocyte globulin	15 related donors, 15 unrelated donors	2 yr	aGVHD 2–4 in 56% cGVHD 75%	23 alive 40% CR, 53% PR OS 72% PFS 67% Non-relapse mortality 15%
Dreger et al, 2003[98] (EBMT)	77	Median no. of previous CT regimens = 3	56% moderate (low dose TBI or flu/cyclo) 44% more intense (flu/bu or high-dose melphelan) 40% had in vivo TCD	HLA-identical siblings in 81%	2 yr		TRM 18% Probability of relapse 31% EFS 56% OS 72% DLI/2nd SCT in 19 with response in 37%
Sorror et al, 2005[99]	64	Advanced CLL	2 Gy TBI with (53) or without (11) fludarabine	44 related donors, 20 unrelated donors	2 yr	Grade 2, 3, 4 aGVHD and cGVHD 39%, 14%, 2% and 50%	Incidence of relapse/ progression 26% Relapse mortality 18% Non-relapse mortality 22% OS 60% DFS 52%
Morris et al, 2005[100]	88	41 LG-NHL 37 HG-NHL, 10 MCL 37 prior autografts; 21 in CR	Alemtuzumab/fludarabine/ melphelan	65 related donors, 23 unrelated donors	36 mo	Grade 3–4 aGVHD in 4 cGVHD in 6	OS 73% 100-d TRM 2% 3-yr TRM 11% PFS 65%

Abbreviations: PBSC, peripheral blood stem cells; aGVHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; CR, complete remission; PR, partial remission; PFS, progression-free survival; TRM, treatment-related mortality; EFS, event-free survival; DFS, disease-free survival; TBI, total body irradiation; TCD, T-cell–depleted; DLI, donor lymphocyte infusion; SCT, stem cell transplant; LG-NHL, low-grade non-Hodgkin lymphoma; HG-NHI, high-grade non-Hodgkin lymphoma; MCL, mantle cell lymphoma.

nificant activity in the relapsed setting.[78,79] Limited data exist on the combination of fludarabine and alemtuzumab, but in one study in patients with refractory disease the combination of fludarabine and alemtuzumab induced impressive responses in patients who were refractory to both of these agents used singly.[80] The CALGB conducted a phase II trial in untreated patients of four cycles of fludarabine followed by alemtuzumab after a 2-month observation period for patients with stable disease or better. Response rates after fludarabine were disappointing (4% CRs and 52% PRs) possibly due to an insufficient number of courses. However, on completion of alemtuzumab, there were 42% CRs and 50% PRs. Eight patients developed cytomegalovirus (CMV) infections, with one fatality.[81] The combination of both rituximab and alemtuzumab has also been studied in relapsed and refractory CLL. Patients had a 63% response rate with CRs observed in 6%, but more than half of the patients experienced an infection.[82]

A number of other monoclonal antibodies are currently in preclinical and early clinical development including anti-CD23 (lumiliximab). This antibody is of interest as expression of CD23 is a characteristic feature of CLL. Other antibodies of potential interest in CLL include anti-CD40, anti-CD22 (epratuzumab), and anti-MHC class II antibodies such as Hu1D10 (apolizumab). A novel approach targeting not the CLL itself but its microenvironment is the use of the anti vascular endothelial growth factor antibody bevacizumab. Another antibody approach in preclinical evaluation is a tetravalent tandem diabody (tanDb) specific for both human CD19 and CD3. Mononuclear cells from CLL patients were cultured with the bispecific antibody fragments, resulting in almost quantitative elimination of malignant B cells by targeting of autologous T cells in 19 of 23 cases.[83]

Immunotoxins are comprised of peptides, usually an antibody or growth factor, that are linked to a toxin such as diphtheria toxin, pseudomonas exotoxin, or ricin. BL22 is an immunotoxin directed against CD22 and fused to pseudomonas exotoxin. It has been used successfully in hairy cell leukemia and is now being investigated as a potential novel agent in CLL.[84] Another agent under investigation is denileukin diftitox (Ontak, Seragen, San Diego, CA). This is a diphtheria–IL-2 immunotoxin that targets IL-2 receptors, which can be found on the surface of around half of the patients with CLL. It induces partial remissions in patients who are refractory to fludarabine but is associated with infusion-related events, a vascular leak syndrome, and elevation of hepatic AST and ALT.[85,86]

Stem Cell Transplantation and the Graft-Versus-Leukemia Effect in CLL

Allogeneic stem cell transplantation (alloSCT) represents the one area where inducing an immune response against tumor cells has been particularly successful. There is strong evidence of a graft-versus-leukemia (GVL) effect in CLL, which can be induced by donor lymphocyte infusions (DLI) or withdrawal of immunosuppressive drugs.[87–89] Historically, alloSCT has been associated with high treatment related mortality in CLL.[90] However, alloSCT may have curative potential in CLL and sustained remissions in chemo-refractory patients may be achieved. There is also evidence to suggest that minimal residual disease (MRD) post alloSCT can be eliminated with DLI even in patients with unmutated IgV_H genes, suggesting that long-term disease-free survival post alloSCT is immunologically mediated.[91,92]

In an attempt to reduce the high treatment-related mortality (TRM) associated with conventional myeloablative conditioning regimens while maintaining the advantages of GVL effect on tumor control, reduced intensity conditioning regimens (RIC alloSCT) have been designed. These generally use fludarabine or low-dose total body irradiation (TBI) for conditioning and conventional immunosuppressive agents for graft-versus-host disease (GVHD) prophylaxis. They also add some form of host and donor T-cell depletion with antilymphocyte globulin or alemtuzumab. Initially it was reported that the GVHD risk was low because of the effect of fludarabine on host DCs (thought to important for the generation of GVHD).[93]

RIC alloSCT has been compared with standard conditioning alloSCT. Of 448 patients with CLL, 228 received RIC and 222 received standard conditioning. The results provided a hazard ratio for TRM of 0.5 (95% confidence interval [CI], 0.3 to 0.83; $P = .007$) and for overall survival (OS) of 0.56 (95% CI, 0.37 to 0.86; $P = .007$), suggesting that the efficacy is as good with RIC while the risks are significantly less. No increased risk of relapse was seen.[94] The results of reported clinical trials of RIC alloSCT are shown in Table 1. The initial optimism about this technique is tempered as non-relapse mortality remains high in older patients with advanced disease and GVHD can result in considerable reduction in quality of life.[95] The GVHD risk has been reduced with T-cell depletion but results in increased risk of post-transplant infections. Long-term follow-up information is still lacking.

Conclusions

A successful immune response to cancer requires tumor antigens that can be presented with appropriate costimulatory signals to T cells able to respond to these antigens. The challenge in CLL is that all three of these facets require work: appropriate tumor antigens remain to be found, antigen presentation and production of costimulatory signals is downregulated in CLL cells, and interactions occur between the malignant cells and the T cells such that the T cells become hyporesponsive. Positive steps have been made but these have yet to translate into potent vaccine development as seen in other malignancies such as malignant melanoma.

Monoclonal antibodies have been shown to be useful and are likely to become standard treatment in the form of combination chemo-immunotherapy. Clinical trials are needed to compare this treatment modality with autologous transplantation as it may be that response rates are as good without the toxic effects of transplantation.

RIC alloSCT with the option for post-transplantation do-

nor lymphocyte infusion is the first real example of the way in which manipulation of the immune system can cure this cancer. Unfortunately this technique still has limited application due to donor availability and toxicity from GVHD. It is certainly not indicated for CLL patients with low-risk disease and is not applicable to the majority of patients who are too elderly to undergo this approach. Further study of how cancer evades autologous immunologic attack and how allogeneic immune cells can override this will produce new targets for intervention.

References

1. Hewitt HB, Blake ER, Walder AS: A critique of the evidence for active host defence against cancer, based on personal studies of 27 murine tumors of spontaneous origin. Br J Cancer 33:241-259, 1976
2. Rosenberg SA, Lotze MT, Muul LM, et al: Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med 313:1485-1492, 1985
3. Gitelson E, Hammond C, Mena J, et al: Chronic lymphocytic leukemia-reactive T cells during disease progression and after autologous tumor cell vaccines. Clin Cancer Res 9:1656-1665, 2003
4. Juffs H, Fowler N, Saal R, et al: B cell chronic lymphocytic leukemia cells have reduced capacity to upregulate expression of MHC class I in response to interferon-gamma. Pathology 36:69-76, 2004
5. Williams JF, Petrus MJ, Wright JA, et al: fas-mediated lysis of chronic lymphocytic leukemia cells: Role of type I versus type II cytokines and autologous fasL-expressing T cells. Br J Haematol 107:99-105, 1999
6. Foa R, Giovarelli M, Jemma C, et al: Interleukin 2 (IL 2) and interferon-gamma production by T lymphocytes from patients with B-chronic lymphocytic leukemia: Evidence that normally released IL 2 is absorbed by the neoplastic B cell population. Blood 66:614-619, 1985
7. Totterman TH, Carlsson M, Simonsson B, et al: T-cell activation and subset patterns are altered in B-CLL and correlate with the stage of the disease. Blood 74:786-792, 1989
8. Tinhofer I, Marschitz I, Kos M, et al: Differential sensitivity of $CD4^+$ and $CD8^+$ T lymphocytes to the killing efficacy of Fas (Apo-1/CD95) $ligand^+$ tumor cells in B chronic lymphocytic leukemia. Blood 91: 4273-4281, 1998
9. Rezvany MR, Jeddi-Tehrani M, Osterborg A, et al: Oligoclonal TCRBV gene usage in B-cell chronic lymphocytic leukemia: Major perturbations are preferentially seen within the CD4 T-cell subset. Blood 94: 1063-1069, 1999
10. Farace F, Orlanducci F, Dietrich PY, et al: T cell repertoire in patients with B chronic lymphocytic leukemia. Evidence for multiple in vivo T cell clonal expansions. J Immunol 153:4281-4290, 1994
11. Goolsby CL, Kuchnio M, Finn WG, et al: Expansions of clonal and oligoclonal T cells in B-cell chronic lymphocytic leukemia are primarily restricted to the CD3(+)CD8(+) T-cell population. Cytometry 42:188-195, 2000
12. Cantwell M, Hua T, Pappas J, et al: Acquired CD40-ligand deficiency in chronic lymphocytic leukemia. Nat Med 3:984-989.
13. Rossi E, Matutes E, Morilla R, et al: Zeta chain and CD28 are poorly expressed on T lymphocytes from chronic lymphocytic leukemia. Leukemia 10:494-497, 1996
14. Gorgun G, Holderried TA, Zahrieh D, et al: Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. J Clin Invest 115:1797-1805, 2005
15. Scrivener S, Goddard RV, Kaminski ER, et al: Abnormal T-cell function in B-cell chronic lymphocytic leukemia. Leuk Lymphoma 44: 383-389, 2003
16. Beyer M, Kochanek M, Darabi K, et al: Reduced frequencies and suppressive function of $CD4^+$ $CD25^{hi}$ regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. Blood 106:2018-2025, 2005
17. Heilmann E, Witting C, Starcke A: Clinical treatment of chronic lymphatic leukemia by means of extracorporeal blood irradiation. Folia Haematol Int Mag Klin Morphol Blutforsch 105:338-341, 1978
18. Muller CP, Treiber D, Steinke B, et al: Enhanced antigenicity of autologous leukemia cells enriched with cholesterylhemisuccinate. Anticancer Res 11:925-930, 1991
19. Ranheim EA, Kipps TJ: Activated T cells induce expression of B7/BB1 on normal or leukemic B cells through a CD40-dependent signal. J Exp Med 177:925-935, 1993
20. Yellin MJ, Sinning J, Covey LR, et al: T lymphocyte T cell-B cell-activating molecule/CD40-L molecules induce normal B cells or chronic lymphocytic leukemia B cells to express CD80 (B7/BB-1) and enhance their costimulatory activity. J Immunol 153:666-674, 1994
21. Van den Hove LE, Van Gool SW, Vandenberghe P, et al: CD40 triggering of chronic lymphocytic leukemia B cells results in efficient alloantigen presentation and cytotoxic T lymphocyte induction by up-regulation of CD80 and CD86 costimulatory molecules. Leukemia 11:572-580, 1997
22. Buhmann R, Nolte A, Westhaus D, et al: CD40-activated B-cell chronic lymphocytic leukemia cells for tumor immunotherapy: Stimulation of allogeneic versus autologous T cells generates different types of effector cells. Blood 93:1992-2002, 1999
23. Krackhardt AM, Harig S, Witzens M, et al: T-cell responses against chronic lymphocytic leukemia cells: Implications for immunotherapy. Blood 100:167-173, 2002
24. Hoogendoorn M, Wolbers JO, Smit WM, et al: Generation of B-cell chronic lymphocytic leukemia (B-CLL)-reactive T-cell lines and clones from HLA class I-matched donors using modified B-CLL cells as stimulators: Implications for adoptive immunotherapy. Leukemia 18:1278-1287, 2004
25. Kater AP, Evers LM, Remmerswaal EB, et al: CD40 stimulation of B-cell chronic lymphocytic leukemia cells enhances the anti-apoptotic profile, but also Bid expression and cells remain susceptible to autologous cytotoxic T-lymphocyte attack. Br J Haematol 127:404-415, 2004
26. Gricks CS, Zahrieh D, Zauls AJ, et al: Differential regulation of gene expression following CD40 activation of leukemic compared to healthy B cells. Blood104:4002-4009, 2004
27. 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. Nat Med 9:279-286, 2003
28. Cheadle EJ, Gilham DE, Thistlethwaite FC, et al: Killing of non-Hodgkin lymphoma cells by autologous CD19 engineered T cells. Br J Haematol 129:322-332, 2005
29. Arditti FD, Aviner S, Dekel B, et al: Eradication of B-CLL by autologous and allogeneic host nonreactive anti-third-party CTLs. Blood 105:3365-3371, 2005
30. Bonyhadi M, Frohlich M, Rasmussen A, et al: In vitro engagement of CD3 and CD28 corrects T cell defects in chronic lymphocytic leukemia. J Immunol 174:2366-2375, 2005
31. Janeway CA Jr, Sakato N, Eisen HN: Recognition of immunoglobulin idiotypes by thymus-derived lymphocytes. Proc Natl Acad Sci U S A 72:2357-2360, 1975
32. Bogen B, Malissen B, Haas W: Idiotope-specific T cell clones that recognize syngeneic immunoglobulin fragments in the context of class II molecules. Eur J Immunol 16:1373-1378, 1986
33. Campbell MJ, Carroll W, Kon S, et al: Idiotype vaccination against murine B cell lymphoma. Humoral and cellular responses elicited by tumor-derived immunoglobulin M and its molecular subunits. J Immunol 139:2825-2833, 1987
34. George AJ, Folkard SG, Hamblin TJ, et al: Idiotypic vaccination as a treatment for a B cell lymphoma. J Immunol 141:2168-2174, 1998
35. Kwak LW, Campbell MJ, Czerwinski DK, et al: Induction of immune responses in patients with B-cell lymphoma against the surface-immunoglobulin idiotype expressed by their tumors. N Engl J Med 327: 1209-1215, 1992
36. Hsu FJ, Caspar CB, Czerwinski D, et al: Tumor-specific idiotype vaccines in the treatment of patients with B-cell lymphoma—Long-term results of a clinical trial. Blood 89:3129-3135, 1997
37. Trojan A, Schultze JL, Witzens M, et al: Immunoglobulin framework-

derived peptides function as cytotoxic T-cell epitopes commonly expressed in B-cell malignancies. Nat Med 6:667-672, 2000
38. Harig S, Witzens M, Krackhardt AM, et al: Induction of cytotoxic T-cell responses against immunoglobulin V region-derived peptides modified at human leukocyte antigen-A2 binding residues. Blood 98:2999-3005, 2001
39. Tao MH, Levy R: Idiotype/granulocyte-macrophage colony-stimulating factor fusion protein as a vaccine for B-cell lymphoma. Nature 362:755-758, 1993
40. Bendandi M, Gocke CD, Kobrin CB, et al: Complete molecular remissions induced by patient-specific vaccination plus granulocyte-monocyte colony-stimulating factor against lymphoma. Nat Med 5:1171-1177, 1999
41. Czuczman MS, Class K, Scheinberg DA: Interleukin-4 priming enhances a target for human complement-mediated cytotoxicity of CLL. Leukemia 7:1020-1025, 1993
42. Siegel S, Wagner A, Kabelitz D, et al: Induction of cytotoxic T-cell responses against the oncofetal antigen-immature laminin receptor for the treatment of hematologic malignancies. Blood 102:4416-4423, 2003
43. Reker S, Meier A, Holten-Andersen L, et al: Identification of novel survivin-derived CTL epitopes. Cancer Biol Ther 3:173-179, 2004
44. Wang Z, Zhang Y, Mandal A, et al: The spermatozoa protein, SLLP1, is a novel cancer-testis antigen in hematologic malignancies. Clin Cancer Res 10:6544-6550, 2004
45. Krackhardt AM, Witzens M, Harig S, et al: Identification of tumor-associated antigens in chronic lymphocytic leukemia by SEREX. Blood 100:2123-2131, 2002
46. Spaner DE, Hammond C, Mena J, et al: A phase I/II trial of oxidized autologous tumor vaccines during the "watch and wait" phase of chronic lymphocytic leukemia. Cancer Immunol Immunother 54: 635-646, 2005
47. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 392:245-252, 1998
48. Brossart P, Bevan MJ: Presentation of exogenous protein antigens on major histocompatibility complex class I molecules by dendritic cells: Pathway of presentation and regulation by cytokines. Blood 90:1594-1599, 1997
49. Brossart P, Wirths S, Brugger W, et al: Dendritic cells in cancer vaccines. Exp Hematol 29:1247-1255, 2001
50. Nestle FO, Alijagic S, Gilliet M, et al: Vaccination of melanoma patients with peptide- or tumor lysate-pulsed dendritic cells. Nat Med 4:328-332, 1998
51. Thurner B, Haendle I, Roder C, et al: Vaccination with mage-3A1 peptide-pulsed mature, monocyte-derived dendritic cells expands specific cytotoxic T cells and induces regression of some metastases in advanced stage IV melanoma. J Exp Med 190:1669-1678, 1999
52. Murphy GP, Tjoa BA, Simmons SJ, et al: Infusion of dendritic cells pulsed with HLA-A2-specific prostate-specific membrane antigen peptides: A phase II prostate cancer vaccine trial involving patients with hormone-refractory metastatic disease. Prostate 38:73-78, 1999
53. Murphy GP, Tjoa BA, Simmons SJ, et al: Phase II prostate cancer vaccine trial: Report of a study involving 37 patients with disease recurrence following primary treatment. Prostate 39:54-59, 1999
54. Kugler A, Stuhler G, Walden P, et al: Regression of human metastatic renal cell carcinoma after vaccination with tumor cell-dendritic cell hybrids. Nat Med 6:332-336, 2000
55. Fong L, Hou Y, Rivas A, et al: Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proc Natl Acad Sci U S A 98:8809-8814, 2001
56. Timmerman JM, Czerwinski DK, Davis TA, et al: Idiotype-pulsed dendritic cell vaccination for B-cell lymphoma: Clinical and immune responses in 35 patients. Blood 99:1517-1526, 2002
57. Goddard RV, Prentice AG, Copplestone JA, et al: Generation in vitro of B-cell chronic lymphocytic leukemia-proliferative and specific HLA class-II-restricted cytotoxic T-cell responses using autologous dendritic cells pulsed with tumor cell lysate. Clin Exp Immunol 126:16-28, 2001
58. Muller MR, Tsakou G, Grunebach F, et al: Induction of chronic lymphocytic leukemia (CLL)-specific CD4- and CD8-mediated T-cell responses using RNA-transfected dendritic cells. Blood 103:1763-1769, 2004
59. Goddard RV, Prentice AG, Copplestone JA, et al: In vitro dendritic cell-induced T cell responses to B cell chronic lymphocytic leukemia enhanced by IL-15 and dendritic cell-B-CLL electrofusion hybrids. Clin Exp Immunol 131:82-89, 2003
60. Kokhaei P, Rezvany MR, Virving L, et al: Dendritic cells loaded with apoptotic tumor cells induce a stronger T-cell response than dendritic cell-tumor hybrids in B-CLL. Leukemia 17:894-899, 2003
61. Hus I, Rolinski J, Tabarkiewicz J, et al: Allogeneic dendritic cells pulsed with tumor lysates or apoptotic bodies as immunotherapy for patients with early-stage B-cell chronic lymphocytic leukemia. Leukemia 19:1621-1627, 2005
62. Mocikat R, Selmayr M, Thierfelder S, et al: Trioma-based vaccination against B-cell lymphoma confers long-lasting tumor immunity. Cancer Res 57:2346-2349, 1997
63. Wahl U, Nossner E, Kronenberger K, et al: Vaccination against B-cell chronic lymphocytic leukemia with trioma cells: preclinical evaluation. Clin Cancer Res 9:4240-4246, 2003
64. 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. Hum Gene Ther 12:659-670, 2001
65. Wendtner CM, Kofler DM, Theiss HD, et al: Efficient gene transfer of CD40 ligand into primary B-CLL cells using recombinant adeno-associated virus (rAAV) vectors. Blood 100:1655-1661, 2002
66. Tolba KA, Bowers WJ, Hilchey SP, et al: Development of herpes simplex virus-1 amplicon-based immunotherapy for chronic lymphocytic leukemia. Blood 98:287-295, 2001
67. 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 105:2436-2442, 2005
68. Wierda WG, Cantwell MJ, Woods SJ, et al: CD40-ligand (CD154) gene therapy for chronic lymphocytic leukemia. Blood 96:2917-2924, 2000
69. Spaner DE, Hammond C, Mena J, et al: Effect of IL-2R beta-binding cytokines on costimulatory properties of chronic lymphocytic leukemia cells: Implications for immunotherapy. Br J Haematol 127:531-542, 2004
70. Ehrlich P: The Croonian Lecture: "On immunity with special reference to cell life." Proc R Soc 66:424-448, 1900
71. McLaughlin P, Grillo-Lopez AJ, Link BK, et al: Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: Half of patients respond to a four-dose treatment program. J Clin Oncol 16:2825-2833, 1998
72. Byrd JC, Murphy T, Howard RS, et al: Rituximab using a thrice weekly dosing schedule in B-cell chronic lymphocytic leukemia and small lymphocytic lymphoma demonstrates clinical activity and acceptable toxicity. J Clin Oncol 19:2153-2164, 2001
73. O'Brien SM, Kantarjian H, Thomas DA, et al: Rituximab dose-escalation trial in chronic lymphocytic leukemia. J Clin Oncol 19:2165-2170, 2001
74. Hainsworth JD, Litchy S, Barton JH, et al: Single-agent rituximab as first-line and maintenance treatment for patients with chronic lymphocytic leukemia or small lymphocytic lymphoma: A phase II trial of the Minnie Pearl Cancer Research Network. J Clin Oncol 21:1746-1751, 2003
75. Keating MJ, Flinn I, Jain V, et al: Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: Results of a large international study. Blood 99:3554-3561, 2002
76. Stilgenbauer S, Dohner H: Campath-1H-induced complete remission of chronic lymphocytic leukemia despite p53 gene mutation and resistance to chemotherapy. N Engl J Med 347:452-453, 2002
77. Byrd J, Peterson B, Park K: Rituximab added to fludarabine improves response in previously untreated chronic lymphocytic leukemia: Preliminary results from CALGB 9712. Proc Am Soc Clin Oncol 20:280a, 2001 (abstr 1116)
78. Keating MJ, O'Brien S, Albitar M, et al: Early results of a chemoimmu-

notherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. J Clin Oncol 23:4079-4088, 2005
79. Wierda W, O'Brien S, Wen S, et al: Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab for relapsed and refractory chronic lymphocytic leukemia. J Clin Oncol 23:4070-4078, 2005
80. Kennedy B, Rawstron A, Carter C, et al: Campath-1H and fludarabine in combination are highly active in refractory chronic lymphocytic leukemia. Blood 99:2245-2247, 2002
81. Rai KR, Byrd JC, Peterson B et al: A phase II trial of fludarabine followed by alemtuzumab (CAMPATH-1H) in previously untreated chronic lymphocytic leukemia (CLL) patients with active disease: Cancer and Leukemia Group B (CALGB) study 19901. Blood 100: 205a, 2002 (abstr 772)
82. Faderl S, O'Brien S: Combined use of alemtuzumab and rituximab in patients with relapsed or refractory chronic lymphoid malignancies: An update of the M.D. Anderson experience. Blood 100:206a, 2002 (abstr 775)
83. Reusch U, Le Gall F, Hensel M, et al: Effect of tetravalent bispecific CD19xCD3 recombinant antibody construct and CD28 costimulation on lysis of malignant B cells from patients with chronic lymphocytic leukemia by autologous T cells. Int J Cancer 112 509-518, 2004
84. Kreitman RJ, Wilson WH, Bergeron K, et al: Efficacy of the anti-CD22 recombinant immunotoxin BL22 in chemotherapy-resistant hairy-cell leukemia. N Engl J Med 345:241-247, 2001
85. LeMaistre CF, Meneghetti C, Rosenblum M, et al: Phase I trial of an interleukin-2 (IL-2) fusion toxin (DAB486IL-2) in hematologic malignancies expressing the IL-2 receptor. Blood 79:2547-2554, 1992
86. Fleming DR, Powell BL, Patrick CL: Diphtheria fusion protein ONTAK therapy of patients with fludarabine refractory chronic lymphocytic leukemia. Proc Am Soc Clin Oncol 19:268a, 2002 (abstr 1071)
87. Jarque I, Palau J, Sanz GF, et al: Delayed complete response after allogeneic bone marrow transplantation in chronic lymphocytic leukemia. Blood 82:1036-1038, 1993
88. Mehta J, Powles R, Singhal S, et al: Clinical and hematologic response of chronic lymphocytic and prolymphocytic leukemia persisting after allogeneic bone marrow transplantation with the onset of acute graft-versus-host disease: Possible role of graft-versus-leukemia. Bone Marrow Transplant 17:371-375, 1996
89. Rondon G, Giralt S, Huh Y, et al: Graft-versus-leukemia effect after allogeneic bone marrow transplantation for chronic lymphocytic leukemia. Bone Marrow Transplant 18:669-672, 1996
90. Michallet M, Archimbaud E, Bandini G, et al: HLA-identical sibling bone marrow transplantation in younger patients with chronic lymphocytic leukemia. European Group for Blood and Marrow Transplantation and the International Bone Marrow Transplant Registry. Ann Intern Med 124:311-315, 1996
91. Ritgen M, Stilgenbauer S, von Neuhoff N, et al: Graft-versus-leukemia activity may overcome therapeutic resistance of chronic lymphocytic leukemia with unmutated immunoglobulin variable heavy-chain gene status: Implications of minimal residual disease measurement with quantitative PCR. Blood 104:2600-2602, 2004
92. Ritgen M, Dreger P, Humpe A, et al: Quantitative PCR demonstrates effective graft versus leukemia activity after allogeneic stem cell transplantation using reduced intensity conditioning in patients with chronic lymphocytic leukemia. Blood 100:854a, 2002 (abstr)
93. Khouri IF, Przepiorka D, van Besien K, et al: Allogeneic blood or marrow transplantation for chronic lymphocytic leukemia: Timing of transplantation and potential effect of fludarabine on acute graft-versus-host disease. Br J Haematol 97:466-473, 1997
94. Dreger P, van Biezen A, Brand R: Reduced-intensity conditioning lowers treatment-related mortality (TRM) of allogeneic stem cell transplantation (SCT) for CLL: A retrospective study on 448 patients. Blood 102:197a, 2003 (abstr)
95. Giralt S, Thall PF, Khouri I, et al: Melphalan and purine analog-containing preparative regimens: Reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood 97:631-637, 2001
96. Khouri IF, Keating M, Korbling M, et al: Transplant-lite: Induction of graft-versus-malignancy using fludarabine-based nonablative chemotherapy and allogeneic blood progenitor-cell transplantation as treatment for lymphoid malignancies. J Clin Oncol 16:2817-2824, 1998
97. 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. J Clin Oncol 21:2747-2753, 2003
98. Dreger P, Brand R, Hansz J, et al: Treatment-related mortality and graft-versus-leukemia activity after allogeneic stem cell transplantation for chronic lymphocytic leukemia using intensity-reduced conditioning. Leukemia 17:841-848, 2003
99. Sorror ML, Maris MB, Sandmaier BM, et al: Hematopoietic cell transplantation after nonmyeloablative conditioning for advanced chronic lymphocytic leukemia. J Clin Oncol 23:3819-3829, 2005
100. Morris E, Thomson K, Craddock C, et al: Outcomes after alemtuzumab-containing reduced-intensity allogeneic transplantation regimen for relapsed and refractory non-Hodgkin lymphoma. Blood 104: 3865-3871, 2004