# Emerging antibody-targeted therapy in leukemia and lymphoma: current concepts and clinical implications

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**The success of immune-mediated therapies has encouraged studies on passive and active immunotherapy in leukemia and lymphoma. This review outlines the impact of increasing insights from basic immunology studies on the potentiation of effective immune responses and the identification of new antigens as targets for antibody (Ab)-targeted therapies. The principles of treatment in leukemia and lymphoma based on current knowledge on the classification of hematologic malignancies are reviewed, and discussed in the context of a rationale to implement new Ab-targeted immunotherapeutic approaches. An update is provided on the use of Ab-targeted therapies in clinical trials with emphasis on new emerging strategies to further expand the successful field of immunotherapy in leukemia and lymphoma.** ***Anti-Cancer Drugs*** **15:189–201**


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## Abbreviations

Abs, monoclonal antibodies; ADCC, antibody-dependent cellular cytotoxicity; Ags, antigens; ALK, anaplastic large-cell lymphoma kinase; alloPSCT, allogeneic stem cell transplantation; AML, acute myeloid leukemia; BM, bone marrow; B-CLL, B cell chronic lymphocytic leukemia; BEAM, BCNU, etoposide, cytarabine, melphalan; CDC, complement-dependent cytotoxicity; CDR, complementarity-determining region; CHOP, cyclophosfamide, doxorubicin, oncovin, prednisone; CHOEP, CHOP with etoposide; CML, chronic myeloid leukemia; CR, complete remission; CRu, complete remission undetermined; CVP, cyclophosfamide, vincristine, prednison; DC, dendritic cell; DHAP, dexamethasone, cytarabine, cisplatin; DLBL, diffuse large B cell lymphoma; DLI, donor lymphocyte infusion; DT, diptheria toxin; EFS, event free survival; EORTC, European Organization for Research and Treatment of Cancer; GvHD, graft versus host disease; HACA, human anti chimeric antibody; HAMA, human anti-murine antibody; HOVON, Dutch and Belgian Cooperative Group in Hemato-oncology; IPI, international prognostic index; IPSS, international prognostic score system; LDH, lactate dehydrogenase; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; NK, natural killer cell; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PMN, polymorphonuclear cell; PR, partial response; PTK, protein tyrosine kinase; R-CHOP, rituximab combined with CHOP; RIC, radio-immunoconjugate; RIT, radio-immunotherapy; SIRS, systemic inflammatory response syndrome; SLL, small lymphocytic lymphoma; T-PLL, T pro-lymphocytic leukemias; TNF-α, tumor necrosis factor-α; VEGF, vascular endothelial growth factor; VIM, VP16, ifosfamide, methotrexate; VOD, veno-occlusive disease; WHO, World Health Organization.

## Introduction

The recent success in the application of engineered Abs in the treatment of B cell non-Hodgkin's lymphoma has contributed to further development of Abs to a variety of leukemia- and lymphoma-associated Ags. This review outlines some basic aspects of identifying potential new targets for Ab-based strategies and clinical implications in developing new treatment regimens for patients with leukemia and lymphoma, without extensively summarizing clinical trials. In developing new immunotherapeutic approaches in the treatment of leukemia and lymphoma, two basic strategies can be distinguished, e.g. active versus passive immunotherapy.

### Active immunotherapy

Active immunotherapy refers to the induction of a specific immune response to malignant cells *in vivo*, i.e. by a vaccination strategy with tumor lysates or with known tumor 'specific' Ags and peptides with or without potentiation of immunogenicity by DCs [1]. New vaccination strategies with DCs to optimize antigen presentation of tumor-specific Ags are being developed [2,3]. This approach would be of most interest in a situation of minimal residual disease, e.g. the development of leukemic DC vaccination programs in AML and CML [4–7]. By utilizing the potential of professional antigen-presenting cells, e.g. DCs, it is possible to present a wide range of (un)determined endogenous leukemic Ags for inducing autologous specific immunity

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after vaccination. Several approaches may be used to optimize the presentation of tumor-specific Ag by DC in active specific immunotherapy programs, e.g. by targeting genetic vectors such as adenoviral gene transfer by novel bispecific single-chain Fv Abs to transduce genes encoding immune-stimulatory molecules such as GM-CSF or with genes encoding molecules involved in antigen presentation e.g. CD40 or CD80 [8,9]. With respect to B cell malignancies, the neoplastic B cells have a unique immunoglobulin which can serve as a target for active immunotherapy. When the idiotype, the unique portion of each immunoglobulin, is used as vaccine, Ab and T cells can be induced to reject the tumor by the host [10]. The method is hampered by the fact that for each patient a different vaccine should be constructed. To further improve the immunogenicity, idiotype protein can be coupled to keyhole limpet hemocyanin and can be used to pulse DCs to optimize T cell reactivity. Finally, the DLIs as cellular therapies in patients undergoing alloPSCT emphasize the major impact of active immunotherapeutic approaches in leukemia and lymphoma.

#### Passive immunotherapy

Passive immunotherapy refers to therapeutic interventions which *modulate* specific and non-specific immune reactivity without the direct induction of specific immunity. The use of Abs recognizing tumor specific Ags has provided an effective modality in the treatment of hematological malignancies. The use of cytokines, e.g. IL-2, IL-12, G-CSF, GM-CSF and interferon-$\alpha$, as well as that of neutralizing Abs, e.g. anti-TNF-$\alpha$ or TNF-$\alpha$ receptor antagonists, may enhance immune effector cell functions or down-modulate dysregulated immune reactivity, e.g. acute and chronic GvHD in alloPSCT. In this review we will focus on the development of Abs in the treatment of hematological malignancies.

## General principles for Ab therapies

For developing therapeutic use of Abs, the following key considerations should be made: immunogenicity of the Ab, selection of the appropriate Ab for optimal binding with human effector cells, and selection of target Ags both for inducing apoptosis and/or modulating growth regulatory signals of malignant cells [11]. Additional obstacles to the success of therapeutic Abs include the varying expression of Ag on tumor cells, reversible binding of Ab to Ag, internalization of the Ag–Ab complex, the poor access of Ab to bulky or hypoxic tumors as well as the rapid clearance of Ab by circulating tumor cells. Certain aspects will be discussed in more detail.

#### Immunogenicity

Immunogenicity of murine Abs was one of the most striking limitations of using therapeutic Abs by the rapid formation of HAMAs which prevented the repeated use of the Abs. Host responses were mainly directed to the murine Fc portion of the Ab and less frequently to the CDR inducing anti-idiotypic Abs. New recombinant technologies have been developed to reduce the immunogenicity of the Ab by engineering chimeric, humanized, primatized and human Abs. Chimeric Abs are genetically engineered by combining the variable region domains of the heavy and light chains of murine Ab, and the constant regions of human IgG, mostly of the $IgG_1$ isotype. The chimeric Ab is less immunogenic, resulting in very low or absent HACA responses. The low HACA responses observed can be further attributed to the immunocompromised status of most patients with hematologic malignancies either at presentation or during treatment. Humanized Abs are fully reshaped Abs in which not only the constant regions, but also the variable regions, are of human origin, except for the specific Ag-binding site. By recombinant technology, synthetic oligonucleotides of the mouse CDR are synthesized and expressed in the human variable region domains.

#### Ab selection

High-affinity binding to target antigen is a key feature of the Ab which influences the dose for optimal Ag saturation and, hence, efficacy. After binding with high affinity, the ability to activate host effector functions such as CDC, opsonization and ADCC as well as to induce apoptosis or inhibit survival signals in the targeted neoplastic cells is of critical importance. The human constant regions of the chimeric or humanized Abs contain sites that interact with complement and the Fc$\gamma$ receptors of several immune effector cells, e.g. PMNs, monocytes, macrophages, NK cells and B cells. The four human IgG isotypes vary in their ability to bind C1q component of complement, and mediate CDC with the highest activity of $IgG_1$ and $IgG_3$ and a low activity of $IgG_2$. $IgG_4$ does not activate complement. *In vitro* data as well as in experiments performed in non-immunodeficient murine models show that C1q and, therefore, classical complement activation is a fundamental element for the therapeutic effects of anti-CD20 (rituximab) [12]. Moreover, the effects of NK cells, PMNs and T cells were dispensable. These data might suggest that the use of Ab with point mutations resulting in increased complement-activating function or bispecific $\alpha$CD20/$\alpha$CD55 molecules are of interest to optimize Ab-targeted efficacy. In addition, poor sensitivity to CDC *in vitro* might predict a poor clinical response, whereas high sensitivity to CDC would only indicate a likelihood of response to Ab, e.g. anti-CD20 treatment [13].

In humans, there are three different classes of Fc receptors that participate in mediating host effector cell functions e.g. $FcR_1$, $FcR_2$ and $FcR_3$. The $FcR_1$ (CD64) has a high affinity binding for monomeric Ab, and is expressed on PMNs, monocytes and macrophages. $FcR_2$ (CD32) has a high affinity for Ab–Ag complexes, and is expressed on PMNs, monocytes and macrophages [14]. The $FcR_3$ (CD16) is highly expressed on NK cells. The

constant regions of human $IgG_1$ and $IgG_3$ have high affinity to all three FcRs. In contrast, human $IgG_4$ has only weak binding capacity to the FcRs.

#### Selection of target antigen

Selection of appropriate Ags for Ab-targeted therapy as well as the level of Ag expression on neoplastic cells is of paramount importance for optimal efficacy. The tumor-specific Ag should be expressed on the neoplastic cells only and not on normal cells. The expression of lineage-specific differentiation Ags in hematological malignancies, e.g. CD20 in B cell NHL and CD33 in AML, provide unique targets for Ab-targeted therapy. Besides the level of Ag expression, a homogeneous expression of the selected 'tumor'-specific Ag on the malignant cells is of importance to lower the risk of tumor escape from Ab therapy. Changes in antigenicity through frequent mutations or the presence of a variant Ag may alter Ag–Ab binding. After binding of the Ab to the Ag a stable irreversible binding should be obtained to enhance the induction of several immune effector cell functions. For conjugated Ab with cytostatic drugs or toxins, the selection of target Ag is based on the specific characteristics that after binding of the Ab, internalization of the Ag–Ab complex results in effective anti-tumor activity. A paradigm in hematological malignancies is the humanized anti-CD33 linked to a semisynthetic derivate of calicheamicin (Mylotarg; gemtuzomab ozogamicin) for the treatment of AML, as discussed below. To further optimize Ab-targeted therapy, the Ag of interest should not be secreted in the circulation, influencing pharmacokinetics as well as early binding of the Ab. An ideal Ag for targeting may be involved in apoptosis or modulate growth-regulating signaling. Blocking of survival signals by Ab binding to growth factor receptors is expected to sensitize cells to killing by chemotherapeutic drugs and other apoptosis inducing therapies [15].

Taken together, an $IgG_1$ chimeric or humanized Ab selected for Ab-targeted therapy is most suitable with respect to high binding affinity to FcRs, and induction of target cell killing by CDC and ADCC with a favorable pharmacokinetic profile and low immunogenicity. By cross-linking of target Ags on the surface of neoplastic cells after FcR-mediated binding with immune effector cells, apoptosis can be induced by Ag mediated signaling. Furthermore, Ag–Ab binding may alter the sensitivity of apoptosis by modulating pro-apoptotic proteins, e.g. bax, and anti-apoptotic proteins, e.g. bcl-2.

## Classification of hematological malignancies and its impact on Ab-targeted therapy

#### Classification of NHL: indolent versus aggressive lymphoproliferative diseases.

With respect to this review we will focus on some aspects of disease entities which are of importance for the rationale of using Ab-targeted therapy.

#### Follicular lymphoma

Follicular lymphoma is a neoplasm of follicle center B cells which has at least a partially follicular pattern and comprises up to 70% of the so-called indolent lymphomas. It affects predominantly adults with a median age of 59 years. Histological grading of the follicular lymphomas in grade 1–2 and grade 3 is important. The clinical course of follicular lymphomas with grades 1–2 is indolent and not usually curable. The clinical course of grade 3 follicular lymphomas is more aggressive and potential curable with intensive treatment programs used in DLBL. Based on the clinical course of the disease, B-CLL and SLL, marginal zone lymphomas (MALT lymphoma, nodal and splenic marginal zone lymphomas) and lymphoplasmacytic lymphoma (Waldenström macroglobulinemia) belong to the indolent lymphomas [16]. Although in indolent lymphomas multiple various treatment regimens are used during the course of the disease, no cure is usually achieved. Immunotherapeutic approaches may prolong survival with a rather low toxic regimen.

#### DLBL

DLBL is a diffuse proliferation of large neoplastic B lymphoid cells and constitute 30–40% of adult NHL in western countries. The median age is in the seventh decade with a broad range. DLBL is aggressive but potentially curable with multiagent chemotherapy based on risk assessment according to the IPI or variants of this scoring system, including, age, WHO performance, stage of the disease, LDH and extranodal sites [17]. Several biological, immunological and genetic prognostics factors are being identified such as the proliferative state, the expression of bcl-2, p53 and ALK, and recently gene expression profiling using microarray techniques [17–19]. As mentioned above, DLBL is curable but the major problems in the treatment of aggressive lymphoma are primary refractory and relapsing disease. The ultimate goals of introducing a new modality treatment such as Ab-targeted therapy for aggressive lymphoma are to increase the percentages of patients achieving CR as well as to prolong the EFS and OS. Based on the clinical course, MCL, mediastinal large B cell lymphoma and Burkitt's lymphoma belong to the group of aggressive lymphomas. Most patients with MCL present with advanced disease with bone marrow involvement, 20–30% are leukemic and extranodal or bulky disease manifestations are often seen. In patients treated with conventional chemotherapy, a median OS of 3–4 years can be reached with a cure rate of less than 10% at 10 years. The role of upfront high-dose chemotherapy followed by peripheral stem cell transplantation is not established and currently under investigation. With respect to this overview other aggressive B and T cell NHL, Hodgkin's lymphoma and post-transplantation lymphoproliferative diseases, although of interest, will not be discussed in detail.

### Classification of MDS and AML

MDS is a group of clonal hematopoietic stem cell disorders characterized by dysplasia and ineffective hematopoiesis in one or more lineages, e.g. erythroid, myeloid or concerning megakaryocytopoiesis, presenting mostly in patients over 70 years of age. Until recently, the French–American–British (FAB) classification was used to subgroup different disease entities within MDS based on the presence of at least two dysplastic hematopoietic lineages, the percentages of blasts cells in the bone marrow and peripheral blood as well as the presence of ringsideroblasts. By introducing the IPSS, several risk groups were identified with different courses of disease and overall survival, e.g. risk of evolution to acute leukemia [20]. The IPSS score is based on the amount of peripheral blood cytopenias, percentage of bone marrow blasts and cytogenetic risk factors. From these data it became clear that patients with MDS with over 20% of bone marrow blast cells may be considered as having AML in the proposed WHO classification [21]. It is beyond the scope of this review to discuss the advantages of the WHO classification for identifying disease entities within the MDS subgroups. New insights into the role of combining morphology, immunophenotype, cytogenetics and molecular biology define several new or better-recognized disease entities for risk-adapted treatment programs. These are also of importance for risk-adapted treatment of AML, and should be implemented in the rationale of combining conventional high-dose chemotherapy and new treatment modalities including Ab-targeted therapies as well as myeloablative and non-myeloablative alloPSCT and matched unrelated stem cell transplantation. The primary goal of Ab-targeted therapy in MDS and AML could be an increase in the percentage of CRs, especially in the older patient group with maintenance of CR by introducing post-remission strategies. The role of Ab-targeted therapy especially labeled with radioisotopes in myeloablative conditioning regimens for hematopoietic stem cell transplantations is of interest to further optimize pre-transplant cytoreductive efficacy.

## Principles of treatment in leukemia and lymphoma; a rationale for Ab-targeted therapy

The choice of treatment as either single-agent or polyagent chemotherapy, and as either single or combined modality will largely depend on the underlying malignancy as well as patient characteristics as age and WHO performance. To optimize the benefits of a new treatment modality e.g. Ab-targeted therapy, sequence and integration in current protocols are of critical importance, dependent on the goals defined, e.g. intention to cure or to palliate the patient. Ab-targeted therapy can be included in an induction phase, during intensification as consolidation therapy with or without autologous or alloPSCT, or as maintenance therapy in a post-remission phase of treatment. In addition, Ab-targeted therapy can be included as part of a treatment program in relapsing disease or in patients with primary refractory disease. Furthermore, dose and time intensity of the different modalities are of increasing interest. Especially with respect to Ab-targeted therapy, the potency of the immune system is of critical importance to amount sufficient immune effector cell functions. Resistance to Ab-targeted therapy by impaired innate or acquired immunity may be due to the underlying disease status or to pre-treatment chemo- and/or radiotherapy. Augmentation of impaired CDC and ADCC by the use of colony-stimulating factors, e.g. G-CSF, GM-CSF and M-CSF, or other cytokines, e.g. interferon-$\alpha$ which affects cellular functions of PMNs, monocytes, macrophages, NK cells as well as B and T lymphocytes may be of substantial relevance to optimize Ab-targeted therapy. In addition, similar drug resistance mechanisms known for chemotherapeutics may in part overlap or induce resistance mechanisms involved in Ab mediated apoptotic signaling routes of neoplastic cells. From these data there is no doubt that optimizing treatment schedules for leukemia and lymphoma, diverse and sometimes conflicting approaches should be taken into account. Several approaches as mentioned above will be discussed below and translated to clinical trials with Ab-targeted therapy in leukemia and lymphoma.

## Tumor-specific antigens for Ab-targeted therapy in leukemia and lymphoma

### Indolent and aggressive B cell NHL and T cell lymphoproliferative diseases

#### *CD20*

By searching for targets for Ab-based therapies in B cell malignancies, the expression of the lineage-specific differentiation Ag, CD20 has provided a unique target with limited heterogeneity [11]. The absence of Ag expression on stem cells allows for the recovery of normal B cells following Ab treatment which leads to the destruction of both malignant and normal B cells. The absence of CD20 expression on plasma cells, resulting in an only slight decrease in immunoglobulins after treatment with anti-CD20, parallels the renewal of normal B cells form the stem cell compartment. The clinical efficacy of Ab targeting with CD20 Abs in NHL will be discussed below.

#### *CD22*

CD22 is a B cell-restricted sialoglycoprotein present in virtually all developing B cells, but detectable on the cell surface only at the mature stages of differentiation. In humans, most circulating IgM- and IgD-positive B cells express surface CD22. In lymphoid tissues CD22 is expressed strongly in follicular, mantle and marginal zone B cells, but weakly in germinal centers. In B cell malignancies, 60–80% of the B cells express CD22 dependent on histologic type. The function of CD22 is

uncertain, but it may play a role as an adhesion molecule. After CD22 ligation with CD22 Abs, CD22 is rapidly internalized. Recently, Ab-targeted therapy with anti-CD22 (epratuzumab), a humanized $IgG_1$ Ab (hLL2) has been administered in patients with NHL [22].

#### *Miscellaneous*

CD23, a low-affinity receptor FcεRII for binding with IgE, is expressed on a subset of normal B cells and malignant counterparts. Anti-CD23 (IDEC-152) is a primatized Ab, made from a primate source with strong similarity to the human Ab without a mouse component [23]. Anti-CD23 binds complement and mediates ADCC *in vitro*. Clinical trials are being conducted. HLA-DR is expressed on a variety of hematopoietic progenitor cells, B cells, activated T cells, monocytes and macrophages. Hu1D10, an anti-HLA-DR Ab (Apolizumab) is an Ab directed against a polymorphic determinant (β chain) of HLA-DR. Until now disappointing results and toxicities has been noted in phase I/II clinical trials [24]. Additional specific Ags are being recognized including CD19 and CD24 for developing Ab-targeted therapy. CD80, a co-stimulatory molecule involved in the regulation of T cellular function by B cells and specialized antigen-presenting cells, such as DCs, is expressed on a variety of B cell lymphomas [25]. Cross-linking of CD80 with CD80 Abs has been shown to inhibit cell proliferation and induction of pro-apoptotic molecules. Recently, clinical trials have been initiated using anti-CD80 (galiximab; IDEC-114) in the treatment of B cell malignancies with or without anti-CD20. Only sparse data are available *in vitro* and *in vivo* demonstrating an interesting role for CD40L (anti-CD154) and anti-CTLA-4 in potentiating immune reactivity via antigen presentation of (malignant) B cells [26,27]. An Ab recognizing Ags not restricted to the B cell lineage, but also recognizing other lymphoid cells, e.g. T cells, is anti-CD52 [28].

The use of Ab-targeted therapy in T cell lymphoproliferative diseases is not yet established, in part due to the lower incidence of these disorders. Potential targets of interest are the CD2, CD3, CD25 and CD52 Ags all expressed on a majority of T cells and T cells in lymphoproliferative diseases. Successful use of anti-CD52 (alemtuzomab; MabCampath®) for the treatment of T-PLL has been reported. Anti-CD52 is also used in conditioning regimens in T cell-depleted alloPSCT [29]. The increasing role of non-myeloablative allogeneic (reduced intensity) stem cell transplantation with HLA-matched related and non-family-related regimens emphasize the role of T cells in graft versus leukemia effects as well as its role in GvHD. To this end, the role of Ab targeting in the treatment of refractory GvHD with Abs targeting T cell Ags, e.g. anti-CD52, anti-CD3 (visiluzumab) and anti-CD2 (siplizumab; MEDI-507), will be of increasing interest in near future [29–31]. To further potentiate Ab-induced cell death, several approaches using bispecific Abs or bispecific single-chain fusion proteins are being developed recognizing tumor-specific Ags and immune effector cells, e.g. anti-CD19/anti-CD3 single chain Ab in B cell NHL [32]. Only sparse clinical data are available.

### Hodgkin's lymphoma

Since the leucocyte activation marker CD30 is highly expressed in the Reed Sternberg cells in Hodgkin's lymphoma interest has increased to use Ab-targeted therapy in primary refractory or relapsing Hodgkin's lymphoma. The expression of CD30 in normal cells is restricted to activated T and B cells, and absent on resting lymphocytes and monocytes, and on normal cells outside the immune system. Activation of CD30 by ligation of CD30 Abs has been implicated in the activation-induced cell death of thymocytes. Several CD30 Abs, e.g. SGN-30, a chimeric CD30 Ab, are being developed showing dose-dependent activity *in vitro* and in a xenograft murine model for Hodgkin's lymphoma [33–35]. Some clinical data using immunoconjugates with saporin toxin showed substantial although transient tumor reduction in some patients with Hodgkin's lymphoma [36]. The role of CD80 and CD87 Abs in Hodgkin's lymphoma is of potential interest, but only sparse data are available.

### MDS and AML

Leukemic blasts in MDS and AML are immunophenotypically well characterized. CD33 is a cell-surface glycoprotein receptor that is specific for myeloid cells. CD33 expression is down-regulated during maturation of the myeloid lineage. CD33 Ag is expressed on approximately 90% of leukemic blasts and leukemic clonogenic myeloid precursor cells as well as on normal myeloid precursor cells, but not on $CD34^+$ pluripotent stem cells [37]. After binding of CD33 Abs, internalization of the Ag–Ab complex is initiated. Several approaches using unconjugated Ab (HuM195) as well as conjugated Ab, e.g. RIC, and immunotoxins have been developed [38,39]. Since CD33 Abs are rapidly internalized, the use as unconjugated Ab-targeted therapy was shown not successful in the treatment of AML. Only in a state of minimal residual disease has anti-CD33 Ab-targeted therapy shown some effects in acute promyelocytic leukemias without severe adverse reactions [40]. The most clinical successful approach using anti-CD33 Ab-targeted therapy is by combining it with an immunotoxin, gemtuzumab ozogamicin (Mylotarg) as discussed below.

CD45, a pan-leucocyte Ag, is of particular interest since CD45 expression is limited to cells of the hematopoietic system and has an approximately 10-fold increased expression per cell as compared to CD33. Furthermore, in contrast to CD33, CD45 is not internalized after

binding to anti-CD45. Targeting a non-internalizing Ag prolongs the exposure of targeted cells and surrounding hematopoietic tissues without intracellular degradation or isotope cleavage, making CD45 suitable especially as a RIC, e.g. in conditioning regimens in autologous and alloPSCT [41].

CD87, a urokinase-type plasminogen activator receptor, is expressed at high density on the surface of approximately 25% of leukemic blasts in AML. The highest expression has been seen in patients with a more aggressive course of their disease [42]. *In vitro* data showed that using the immunoconjugate diphtheria toxin–urokinase fusion protein (DTAT) recognizing leukemic blasts overexpressing CD87, toxic effects as measured by inhibition of proliferation and clonogenic growth of leukemic cells could be measured. Until now no *in vivo* data in mice and humans are available.

Neoangiogenesis has been shown to play an important role in the pathogenesis of MDS and AML. Autocrine and paracrine secretion of angiogenic growth factors such as VEGF in the bone marrow microenvironment may promote proliferation and survival of leukemic blasts. Recently, the VEGF Ab bevacizumab has been shown significant activity in patients with metastatic renal and colorectal carcinoma with respect to time to progression [43,44]. No clinical data are available in patients with MDS and AML, but bevacizumab could be of potential interest. Several new mechanisms are emerging involved in leukemic cell survival and proliferation which might be modified by specific targeting approaches using PTK inhibitors. Small molecules with PTK inhibitory activity such as SU5614 and others inhibits the phosphorylation of VEGF receptors 1 and 2, c-kit Rs and FLT3 (mutated and wild-type), and may therefore modify activation of downstream signaling pathways involved in cell survival and proliferation. In addition, the use of antisense molecules, e.g. a phosphorothioate oligodeoxynucleotide that targets bcl-2 mRNA to form a DNA/RNA duplex (G3139), may specifically alter the sensitivity of leukemic cells to apoptosis. Although of particular interest for developing new multi-modality strategies in the treatment of MDS and AML using such approaches, this review will focus on Ab-targeted therapy.

In particular, in low-risk MDS, an increase in apoptosis of hematopoietic progenitors might play an important role in the pathogenesis of the disease. Several studies have shown increased levels of TNF-α in the bone marrow of patients with low-risk MDS as compared to patients with advanced stages of the disease. Infliximab (Remicade), an anti-TNF-α chimeric Ab and Etanercept (Enbrel), a recombinant soluble TNF-α receptor fusion protein, TNF-αRp75:Fc, have shown some efficacy in patients with low-risk MDS by modifying the availability of TNF-α to induce apoptosis of highly susceptible hematopoietic precursor cells [45]. In addition, neoangiogenesis in MDS reflected by an increased microvessel density in specific subgroups of MDS might be of additional interest to Ab-targeted therapy with anti-VEGF as part of a multimodality regimen including PTK inhibitors as discussed above and/or angiogenesis inhibitors such as thalidomide. The role of growth factors, e.g. erythropoietin and G-CSFs, to interfere with increased susceptibility of apoptosis of hematopoietic progenitor cells may be part of a treatment program in subgroups of MDS [46]. The role of TNF-α neutralizing Ab either by an anti-TNF-α chimeric Ab or soluble TNF-α receptor fusion protein in acute and chronic GvHD in hematological malignancies will be discussed below.

## Conjugated Ab-targeted therapy

Immunoconjugated Abs deliver specifically cytotoxic agents to the targeted neoplastic cells. Abs can be conjugated with cytostatic drugs such as calicheamicin or with toxins such as DT, pseudomonas exotoxin (PE38) or deglycosylated ricin A [47]. Ab-bound chemotherapy or toxin is delivered to the interior of the cell through internalization of the Ag–Ab complex. The subsequent impairment of protein synthesis or induction of double-stranded DNA damage results in cell death. Several mechanism of resistance to unconjugated Ab-targeted therapy have been discussed above including insufficient binding of the Ab, lack or less of Ag expression on neoplastic cells, poor access of Ab to the tumor cells or failure of host immune effector system either due to the disease or induced by previous cytotoxic regimens. To circumvent most of these issues, RICs, Abs to which a radioisotope is attached, have been developed [48]. Since both myeloid and lymphoid cells are inherently sensitive to radiation therapy, RICs may provide targeted radiation therapy, not only to the cells that bind the Ab, but, due to bystander or crossfire effects, also to neighboring cells inaccessible to Ab or with insufficient antigen expression. The selection of the isotope for RIT has clinical implications and is dependent on the type of neoplasia and the proposed treatment regimen, e.g. as part of an initial treatment, as maintenance treatment, as salvage therapy or as part of a conditioning regimen in autologous or alloPSCT. The most successful radionucleotides used for RIT in leukemia and lymphoma are the β particles emitted by $^{131}I$ and $^{90}Y$ which are cytotoxic over many cell diameters. $^{131}I$ has been used as radionuclide most often for its low cost, wide accessibility and marked clinical efficacy. $^{90}Y$ is the second most commonly used radionuclide. Its particles are 5 times more energetic that those from $^{131}I$, emit very few γ-rays, have a favorable half-life of 2.5 days and are stably retained by the cells, and hence can be used in an outpatient setting.

## Clinical data on Ab-targeted therapy in leukemia and lymphoma

It is beyond the scope of this review to summarize all available data from clinical trials with Ab-targeted therapy in leukemia and lymphoma. Several excellent reviews were published recently [11,41,47–50]. We will only briefly summarize the conclusions drawn from several studies with major clinical impact, and discuss current questions in designing new treatment modalities to further improve the ultimate outcome in patients with leukemia and lymphoma.

### Indolent NHL

The first clinical trials with anti-CD20, a chimeric Ab [rituximab: Rituxan (US); Mabthera (EU)] were conducted in indolent follicular relapsed or refractory B cell NHL as single-agent from 1 dose to 4 times weekly at a dose of 375 mg/m$^2$. In the pivotal trial, an ORR in the evaluable patients (151 of 166) was 50% with 6% CRs and a median time to progression of 13.1 months [51]. Significant lower responses were seen in SLL versus follicular NHL, in stage IV disease including BM involvement as well as if two or more extranodal sites were involved. No significant impact on response could be seen dependent on age, LDH, $\beta_2$-microglobulin and bulky disease in this heavily pre-treated patient group. Recovery of B cells starts between 6 and 9 months to fully normal within 1 year. The mean levels of IgG and IgA remain normal with only a decrease below normal of IgM at 6 months with full recovery after 8 months. HACA responses were noted in only one patient. Significantly higher serum levels of anti-CD20 were detected in patients with response. Adverse events of grade 1 and 2 were frequently noted during the first infusions and were reduced after each infusion thereafter. Grade 3 and 4 adverse events, e.g. neutropenia, thrombocytopenia, chills and anaphylactoid reactions, were documented in approximately 5% of all reported events. In several clinical trials similar adverse events were identified with sporadically a life-threatening tumor lysis syndrome due to either anti-CD20-induced lymphocyte agglutination in lungs, liver and spleen or a SIRS-like syndrome with lysis of tumor cells. Re-treatment with rituximab was feasible with an ORR of 40% with 11% CR in 57 out of 60 evaluable patients [52]. No increase in HACA responses was noted and a safety profile comparable to that seen in studies using rituximab for the first time. The estimated median duration of response was 16.3 months with a median time to progression of 17.8 months which was comparable or even better to prior course of rituximab treatments. After these trials, several trials optimize treatment schedules by evaluating the number of courses up to 8 times weekly in recurrent indolent NHL. The efficacy of front-line treatment with rituximab followed by maintenance therapy, e.g. repeated 4-week courses every 6 months during 2 years, showed up to 65% ORR with a minimum follow-up of 15 months and a median time to progression which had not been reached [11,53–55].

After the use of rituximab as single-agent, single-modality treatment in indolent NHL in primary refractory, relapsing disease, as front-line therapy with or without maintenance programs, combined modality approaches were evaluated. Since Ab-based targeted therapy differs in mechanisms of actions as described in detail above with a possible lack of cross-resistance mechanisms, combination with conventional chemotherapy could be of interest [11,56,57]. Several trials combining rituximab with or without anthracycline-containing or fludarabine-containing regimens were conducted. ORR rates are as high as 90–100%. In untreated patients with indolent lymphoma using rituximab with standard CHOP (R-CHOP) an ORR of 100% was reached with 63% CR [57]. The median duration of response has not been reached at 63.6 months after a median observation of 65.1 months. The adverse event profile of R-CHOP was consistent with that from each therapy alone. The prolonged PFS of 65.0 months exceeds that reported for CHOP alone. To combine the several approaches discussed above, the HOVON and EORTC study groups have conducted a phase III prospective randomized controlled clinical trial in patients with stage III–IV follicular lymphoma at initial diagnosis who have relapsed after a maximum of two non-anthracycline-containing regimens comparing CHOP versus R-CHOP. In addition, in patients having achieved good PR or CR after remission induction, patients will be randomized to maintenance treatment with anti-CD20 or no further treatment to compare PFS in both groups.

Recently, CD22 Ab-targeted therapy with epratuzomab, a humanized $IgG_1$ Ab (hLL2) has been administered as four once-weekly infusions across a wide range of doses to patients with various indolent NHL histologies [58]. These data showed that epratuzomab has an acceptable safety profile, but exerted only responses in follicular lymphomas (pre-treated with at least one prior regimen; median 3.5; range 1–10 regimens) with a median duration of response of 80 weeks with a median time to progression for responders of 87 weeks by Kaplan–Meier estimates. Combination regimens including chemotherapy and rituximab with epratuzumab with potential non-resistance mechanisms of action are currently under investigation both in indolent and in aggressive NHL. Finally, a recombinant immunotoxin containing an anti-CD22 variable domain (Fv) fused to truncated pseudomonas exotoxin (BL22) administered in patients with hairy cell leukemia resistant to purine analogues showed a CR rate of 69% [59]. During a median follow-up of 16 months, three of 11 patients relapsed, but after re-treatment with BL22 had a second CR. The potential role of BL22 in other indolent lymphomas/leukemias is currently under investigation.

### Ab targeting in B-CLL and lymphoplasmacytoid lymphoma

Until now B-CLL remains incurable, similar to the other indolent lymphomas, despite of decades of clinical trials. In contrast to the follicular lymphomas, CD20 is expressed on B-CLL cells with marked heterogeneity and with low density. Several reports have documented ORR up to 75% with sporadic CR with median overall response durations below 1 year as used as a single regimen with rituximab alone [60,61]. Since Abs might sensitize tumor cells to the effects of subsequent chemotherapy as discussed above, several small studies show the additive effects of combining fludarabine and rituximab either concurrent or sequentially dosed with an ORR of 90 and 77%, respectively, including 47 and 28% CR, but with only a short follow-up period [11,60]. In addition, studies have been performed using rituximab together with fludarabaine and cyclofosfamide showing CR rates up to 66% of the patients with only marginal follow-up. Upfront treatment with standard courses of rituximab alone with a maintenance program of 4-week courses at 6-month intervals has shown a median PFS of 18.6 months and 1- and 2-year PFS rates of 62 and 49%, respectively [62]. Toxicities in B-CLL using rituximab are more frequently seen than in patients with follicular lymphomas, possibly related to the amount of circulating tumor cells.

CD52 is expressed on virtually all lymphocytes at various stages of differentiation as well as on monocytes, macrophages and eosinophils. The function of CD52 Ag is currently unknown. The highest level of expression is on T-PLL cells followed by B-CLL and lowest on normal B cells. Alemtuzomab is a humanized anti-CD52 Ab. After ligation of CD52 by anti-CD52 its cytolytic effect is mediated by ADCC and CDC similar to the action of anti-CD20 (rituximab). In B-CLL, several clinical phase II trials reported responses in previously treated patients with fludarabine and alkylating agents. The ORR in several studies is between 33 and 89% with CR observed from only 4 to 50% in small series with a median duration of response of approximately 8 months. Alemtuzumab is associated with a number of toxicities, some of which are acute and related to the infusion, and others that are more delayed and related to myelosuppression and immunosuppression. Prophylactic treatment for opportunistic infections with pneumocystic carinii pneumonia and reactivation of cytomegalovirus should be considered. The role of alemtuzumab in the treatment of B-CLL is currently being studied in various phase II trials, as single agent in first-line treatment, and as part of multimodality regimens including rituximab and conventional cytostatic drugs such as cyclofosfamide and fludarabine upfront or in recurrent disease [63].

Finally, in lymphoplasmacytoid lymphoma, some data indicate that anti-CD20 Ab-targeted therapy can be used successfully [64]. Especially in the elderly, Ab-targeted therapy with anti-CD20 has a safe toxicity profile with respect to infusion related morbidity and marginal myelosuppresive activity as compared to chlorambucil and fludarabine.

### Radioimmunotherapy in indolent B cell NHL

Recently, excellent reviews have been published dealing with RIT in NHL [49,65]. We will briefly summarize some important issues. The most widely studied of the current generation of RIC in patients with NHL are directed against CD20, linked to either $^{131}$I or $^{90}$Y, e.g. [$^{131}$I]tositumomab (Bexxar) and [$^{90}$Y]ibritumomab tiuxetan (Zevalin) [66–68]. [$^{90}$Y]Ibritmomab tiuxetan is composed of the murine parent of rituximab, ibritumomab (Y2B8), an $IgG_1$ Ab with tiuxetan which functions as a bifunctional chelating reagent with stable retention of $^{90}$Y. [$^{131}$I]Tositumomab is composed of the murine tositumomab (B-1) Ab which is an $IgG_{2a}$ specific for CD20 to which $^{131}$I is linked by a chemical bond. Zevalin is the first RIC to be approved by the FDA for the treatment of relapsed or refractory follicular (indolent) and transformed NHL including rituximab-refractory follicular NHL followed only recently by Bexxar. In a randomized controlled trial of [$^{90}$Y]ibritumomab tiuxetan versus rituximab for patients with relapsed, refractory low-grade, follicular or transformed B-NHL, [$^{90}$Y]ibritumomab tiuxetan was significantly more active with an ORR of 80% compared to 56% for rituximab, 34 CR/CRu with RIC and 20% with rituximab [69]. No difference in response duration could be observed although the time to the next therapy was not yet reached for RIC compared to 15.2 months for the rituximab group. In addition, [$^{90}$Y]ibritumomab tiuxetan is also active in bulky disease as well as in patients pre-treated with rituximab. Toxicities related to [$^{90}$Y]ibritumomab tiuxetan are mainly hematologic of which the nadir occurs, unlike chemotherapy, at about 7–9 weeks following therapy. [$^{131}$I]Tositumomab showed similar results in phase II clinical trials with ORR of 57–97% with CR/CRu between 14 and 63% in follicular relapsed and refractory B-NHL correlated with the extent of and responsiveness to prior treatment [49]. The median PFS in responding patients was between 8 and 19 months. In one study the PFS at 3 years was 68% [67]. The major toxicities are hematologic as is the case for [$^{90}$Y]ibritumomab tiuxetan. For both RICs, secondary AML and MDS are of real concern, being reported to occur in up to 6.3% of patients. Its occurrence seems to be related to prior chemotherapy. The precise role of RICs in the treatment of B-NHL, as initial therapy, as part of a salvage regimen or as maintenance therapy is still under investigation. The use of RICs with potential adverse effects like MDS and the lack of data demonstrating prolongation of survival make its use as initial therapy uncertain. The HOVON and EORTC collaborative study groups are currently performing a randomized prospective trial comparing [$^{90}$Y]ibritumo-

mab tiuxetan versus no further treatment in patients with follicular indolent NHL as post-remission therapy after 8 cycles of standard CVP.

### Aggressive NHL: upfront Ab-targeted therapy in diffuse large B cell NHL

Aggressive DLBL in elderly has a CR rate of approximately 30–40% with a 3-year EFS and OS of 30 and 35%, respectively. Until now, no more efficacious treatment regimens have been identified than CHOP. In phase II trials, it was shown that in DLBL the addition of rituximab to CHOP was feasible with an increase in ORR including CR and an increase in the OS and PFS in patients with high IPI both in *de novo* as well as in relapsed patients with DLBL [70]. In a recently published randomized trial comparing standard CHOP with R-CHOP in an elderly population stratified by age-adjusted IPI with previously untreated DLBL stage II–IV, a significant increase in CR rate was achieved of 63 versus 76%, respectively [71]. Clinical relevant toxicity was not significantly greater with R-CHOP and rituximab did not compromise the dose intensity of CHOP. EFS was significantly longer in the R-CHOP of 57 versus 37% in CHOP alone with an increase in OS at 24 months of 70 versus 57%, respectively. The benefit of R-CHOP was consistent across all subgroups of patients tested, including good and poor risks according to IPI and independent of under 70 year and over 70 years of age. A recent update of these data at 3 years OS confirms the superior results of R-CHOP versus CHOP with OS of 62 versus 51%, an EFS rate of 53% in the R-CHOP versus 35% in CHOP alone (not published). In addition, R-CHOP overcomes bcl-2-associated resistance to chemotherapy in elderly patients with DLBL [72]. In an ongoing international trial comparing R-CHOP versus CHOP alone in patients under 60 year of age and good prognosis (age-adjusted IPI 0 or 1) the additional benefits of rituximab should be demonstrated in the next years. Of interest are studies using CHOP-14 versus CHOP-21 without rituximab in both arms in an elderly patient group with DLBL in which CHOP-14 with G-CSF showed comparable results as obtained for R-CHOP in a standard 3 weekly schedule with a comparable toxicity profile [73]. In the same trial, CHOEP-14 and CHOEP-21 were included. For patients under 60 year of age the addition of etoposide was superior over CHOP-14 and CHOP-21 [74]. The addition of etoposide showed an increase in toxicity in the older patients group. From these data it is of interest whether Ab-targeted therapy, e.g. rituximab, in DLBL combined with a dose-intensity CHOP regimen (R-CHOP-14) is superior over CHOP-14 with respect to EFS, CR, OS as well as toxicity profile. Combining Ab-targeted therapy with increasing dose-intensity regimes supported by e.g. G-CSF, will ultimately improve the cure of patients with DLBL, which is currently under study in the HOVON study group.

### Aggressive NHL: upfront high-dose chemotherapy with Ab targeting in MCL

As described above, upfront high-dose chemotherapy with stem cell rescue is currently under investigation to improve the CR and OS in patients with MCL. It has been shown that mobilizing treatment for stem cell transplantation may increase the number of circulating lymphoma cells and graft contamination especially in MCL [75]. More effective treatment, e.g. purging methods, as well as induction therapy to enhance CR before autografting of a tumor-free stem cell preparation is needed. Recently, a multicenter, retrospective study of patients with MCL treated with high dose chemo-immunotherapy and an *in vivo* purged autologous stem cell graft showed a 100% CR with a continuous CR of 88% after a median follow-up of 35 months [76]. The OS and EFS rates at 54 months were 89 and 79%, respectively, as compared to historical control patients with an OS of 42 and 18% EFS. These data should be confirmed in a multicenter prospective study like the one which is currently performed by HOVON studying three courses of R-CHOP-21 followed by high-dose cytarabine and stem cell mobilization during treatment with rituximab before autografting with a BEAM conditioning regimen in responsive patients.

### Aggressive NHL: Ab targeting in relapsing disease

The 5-year survival of patients with relapsing or refractory DLBL treated with conventional chemotherapy is poor. High-dose chemotherapy and autologous stem cell transplantation regimens improve prognosis and is the treatment of choice, but a substantial proportion of patients are ineligible because of age, WHO and failure to respond to salvage therapy. In phase II trials, the addition of rituximab to regimens containing etoposide, prednisone, vincristine, cyclofosfamide or ifosfamide and carboplatin/cisplatin) has improved response rates in heavily pre-treated patients who relapsed or were primary refractory [77]. In a randomized phase III study, the additional benefit of rituximab during sequential chemotherapy of DHAP–VIM–DHAP followed by autologous stem cell transplantation in patients with relapsed and primary refractory aggressive NHL is currently under evaluation by HOVON to assess the PR and CR before autologous stem cell transplantation and its impact on OS and EFS.

### MDS and AML

The most clinical successful approach using CD33 targeted therapy in myeloid malignancies is the use of gemtuzumab ozogamicin (Mylotarg) [39,78,79]. This is a humanized CD33 Ab linked to a semisynthetic derivate of calicheamicin which is a potent cytotoxic antibiotic. After Ag–Ab internalization, calicheamicin is released inside the lysosomes of myeloblasts, binding to DNA, and causing DNA double-strand breaks and ultimately cell death. After infusion of gemtuzumab ozogamicin nearly

complete saturation of CD33 antigenic sites was reached within 3–6 h for AML blasts, monocytes and PMNs, whereas no binding of gemtuzumab ozogamicin was found on lymphocytes. After rapid internalization of the Ag–Ab complex it was shown that the expression of CD33 was continuously renewed, which can significantly increase the internalization process and thereby the intracellular accumulation of the drug [37]. Finally, it was shown that gemtuzumab ozogamicin induces apoptosis of myeloblasts *in vitro*. Furthermore, expression of the multidrug resistance features of AML cells *in vitro* was correlated with resistance for gemtuzumab ozogamicin and could be modulated by the P-glycoprotein antagonist cyclosporine, suggesting that treatment trials combining gemtuzumab ozogamicin with multidrug resistance-modifying agents is warranted [80].

Several phase II trials in patients with AML in first relapse showed acceptable toxicity with an OR of 30% including patient with CR and CR without recovery of normal, e.g. above $100 \times 10^9$/l, thrombocyte counts [78,79]. Most schemes administered gemtuzumab ozogamicin as a 2-h infusion at 9 mg/m$^2$ for two doses with a 14-day interval at the outpatient department. Older age and shorter remission duration were associated with an inferior response rate with gemtuzumab ozogamicin. An infusion-related symptom complex of fever, chills and hypotension are commonly seen which might be related to cytokine release from myeloblasts, and appeared to be less common in subsequent infusions. The major concern using gemtuzumab ozogamicin is the association with hepatotoxicity including hepatic VOD [81,82]. Hepatotoxicity may be more frequently seen in patients after hematopoietic stem cell transplantation and caution is warranted in patients with multiple risk factors for VOD.

Several RICs has been studied so far for its use in AML [41]. $^{131}$I-labeled murine anti-CD33 was used as part of a conditioning regimen in patients undergoing hematopoietic stem cell transplantation. Since $^{131}$I is a β particle emitter that can deposit energy over 2.4 mm of its vicinity, severe pancytopenias are induced. However, leukemic cells with no or low expression of CD33, which are likely to otherwise escape Ab-targeted therapy, may be destroyed. It is apparent that such therapies would not be feasible without stem cell rescue. Studies with an α particle emitter with a path length of only 5–8 μm such as $^{213}$Bi linked to HuM195 (anti-CD33) may allow the RIC to more selectively destroying leukemic cells whilst preserving surrounding tissues. The use of $^{131}$I linked to an anti-CD45 pan-leucocyte marker could be an alternative Ab-targeted strategy to destroy leukemic clonogenic cells without sparing of normal hematopoietic cells and microenvironment which is only suitable combined with stem cell transplantation. In addition, the use of $^{90}$Y-labeled HuM195 has been studied, a high-energy β emitter without γ emissions labeled with the humanized CD33 Ab showing its feasibility in relapsed and refractory AML patients, thereby suggesting a potential role as part of pre-transplant conditioning regimens.

Several approaches can be defined to further optimize the use of Ab-targeted therapies in high-risk MDS and AML. Since gemtuzumab ozogamicin has been shown to exert a manageable safety profile and is associated with a modest degree of non-hematological toxicity, it can be used safely in the elderly and on an outpatient basis. Further investigation should be focused on the use of gemtuzumab ozogamicin with conventional drugs in remission-induction schedules as well as in remission-maintenance regimens. Its low immunogenicity makes it use suitable for repeated dosing. The HOVON/SAKK study groups have initiated a study in which patients older than 60 years of age in first CR of AML are randomized between no further post-induction therapy or maintenance therapy with 3 cycles of gemtuzumab ozogamicin every 4 weeks at a dose of 6 mg/m$^2$. The use of RICs in high-risk MDS and AML using anti-CD33 and/or anti-CD45 Abs are of specific interest in developing effective conditioning regimens in hematopoietic stem cell programs to further enhance EFS.

### Acute and chronic GvHD in allogeneic stem cell transplantation

Acute GvHD is a serious complication after alloPSCT in which TNF-α plays a central role in the inflammatory cascade of acute GvHD [83,84]. TNF-α can be blocked by TNF-α Ab (infliximab) or neutralizing soluble TNF-α receptors (Etanercept). Several small series of patients and case reports indicate that in therapy-resistant acute and chronic GvHD this approach may be useful in selected cases of patients with especially GvHD of the intestines [85,86]. Caution is recommended when using anti-TNF-α in GvHD since anti-TNF-α administration is associated with a significant increase in risk of non-Candida invasive fungal infections. The use of CD2, CD3 and CD25 Abs in the treatment of GvHD is also of potential interest, but only sparse data are available.

## Future perspectives

The success of immune-mediated therapies has encouraged studies on passive and active immunotherapy in leukemia and lymphoma. Increasing insights from basic immunology studies on the potentiation of effective immune responses and the identification of new Ags as targets for Ab-mediated therapies have further stimulated translational research on developing effective immunotherapeutic strategies. Promising new approaches will combine classical dose-intense chemotherapy with 'tumor-specific' Ab targeting in several phases of the disease. Active specific immunotherapies, e.g. by vaccina-

tion with genetically modified leukemic cells, leukemic dendritic cells, DNA vaccines or peptides as well as adoptive transfer of tumor specific T cells, are emerging especially in minimal residual disease. Allogeneic immunotherapy with reduced intensity conditioning regimens and improved strategies in controlling graft-versus-host disease may further contribute to successfully expand the field of immunotherapy in leukemia and lymphoma.

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