New Development in Monoclonal Antibody Targeting Therapy for Malignant Hematological Disease

Yunbi Fu

Department of Hematology, the First Hospital Affiliated to Shantou University, Shantou 515041, China.

Summary In the past decades, with the development of chemotherapy and stem cell transplantation, increased survival of leukemia has been achieved in patients younger than 55 years, although without significant survival impact in older individuals. Unfortunately, many patients, regardless of age at diagnosis, will eventually die from their disease or the side effects of therapy. Advances in the development of targeted therapies using monoclonal antibody have proven benefit for leukemia and lymphoma. The fusion of a murine B cell and a myeloma cell generates a hybridoma that produces monoclonal antibody (mAb). These murine mAb induce the HAMA (human anti-mouse antibodies) response. Murine mAb have been modified by genetic engineering, producing molecules with a higher proportion of human protein. At present, chimeric, humanized and fully human mAb are available. MAb block interactions between target molecules and their ligands or trigger the lyses of mAb-coated tumor cells. Numerous mAb have been developed using the recombinant DNA technology and several are available in the market.

Key words monoclonal antibody; targeting therapy; leukemia; lymphoma

The availability of antibodies reactive with antigens expressed only by hematopoietic cells has provided new tools to develope new therapies for malignant hematological diseases. Studies up to now have investigated the use of such antibodies in an unmodified state, combined with potent chemicals to form immunotoxins or combined with various radionuclides. In recent years preclinical and clinical studies have been undertaken with selected monoclonal antibodies (MAbs) in patients with several hematologic malignancies, including acute myeloid leukemia(AML), chronic lymphocytic leukemia (CLL), prolymphocytic leukemia (PLL) and lymphoma. Encouraging results have been obtained in all the three settings: unconjugated MoAb, MoAb conjugated with chemotherapy or toxins, and MoAb conjugated with radioisotopes (Table 1). For examples: MAb directed against CD20 antigen (Rituximab, RIT) and radiolabeled CD20 (Zevalin and zevalin) have been used in B cell lymphoma successfully; MAb directed against CD52 antigen (Campath-1H, alemtuzumab, ALT) demonstrate significant activity in CLL; Antibody-targeted chemotherapy with gemtuzumab ozogamicin (GO, Mylotarg), a humanized anti-CD33 antibody conjugated with calicheamicin, is a clinically validated therapeutic option for patients with acute myeloid leukemia (AML).

Correspondence to: Yunbi Fu.
Email: hnfyb@yahoo.com.cn.
Tel: 13929692952

Unconjugated monoclonal antibody

Many unconjugated monoclonal antibodies targeting leukemia/lymphoma are under researching, whether humanized or non-humanized. Unconjugated antibodies have a complex mechanism of action, dependent on the nature of the target structure. Antibodies can activate the immune system (antibody-dependent cellular cytotoxicity [ADCC], complement-dependent cytotoxicity [CDC], induction of tumor immunity [idiotype network]). ADCC appears to be one of the most important immune effector functions. Antibodies may also induce apoptosis, cell cycle arrest, inhibition of cell proliferation as well as angiogenesis and metastatic spread. For most antibodies there is no clear dose-response relationship in vivo. The effect of antibodies can be enhanced by combination with chemotherapy and/or by agents which activate the immune system. The best therapeutic effect may be obtained if mAbs are used ear-

ly in the course of the disease[1]. Anti−CD20 is used for B cell chronic lymphocytic leukemia (CLL)/lymphoma, anti−CD33 for acute myeloid leukemia (AML), anti−CD52 for T cell or B cell lymphoma and CLL and anti−CD2 for T cell leukemia. Some of them are currently commercial and clinical available.

Anti−CD20 (Rituximab, RIT)

Rituximab (RIT) is a high−affinity chimeric mouse anti−CD20 MoAb, with the FDA approval in 1997, which has been major treatment advances for patients with B−cell non−Hodgkin´s lymphoma (NHL). Rituximab produces responses in approximately 50% of cases of relapsed, low grade NHL, with a median duration of these responses of approximately 13 months[2]. Now this drug is in widespread use, and Rituximab combined with CHOP chemotherapy is considered as the most effective regimen for in follicular and high−grade lymphomas. A number of different lymphoid malignancies have been tested for sensitivity to rituximab. Significant, but lower, single−agent response rates have been seen in diffuse large cell lymphoma, mantle cell lymphoma, chronic lymphocytic leukemia (CLL), Waldenstrŏm´s macroglobulinemia, and post−transplant lymphoproliferative disease[3~6].

Several studies have shown synergy between RIT and chemotherapeutic agents. Demidem *et al.* first suggested that RIT pretreatment sensitized chemo−resistant $CD20^+$ lymphoma cell lines to chemotherapeutic agents *in vitro*[7]. Interactions between RIT and purine nucleoside analogues (PNA), fludarabine (FA) and cladribine (2−CdA) also have important practical value. Pretreatment with FA or RIT and complement of the follicular lymphoma cell line Karpas 422 caused cell lysis in 10%~20%. However, the cell lysis increased to 70% when the cells were incubated with both cytotoxic agents[8].

The CD20 antigen is expressed on almost all B−cells in patients with B−CLL but the intensity of expression appears to be lower than that in patients with NHL. Expression of this antigen on B−CLL cells is only 20%~30% of the level found in patients with follicular lymphoma [9]. Moreover, CD20 surface antigen levels are lower in bone marrow and lymph node than that in peripheral blood of CLL patients, and higher count of circulating leukemic B−cells may result in unfavorable pharmacokinetics of RIT. In addition, significant levels of circulating CD20 (cCD20) can be detected in the plasma of patients with CLL. This cCD20 interferes with the binding of RIT. The results of some studies indicate that the cCD20 level is of prognostic significance [10]. At present it is not clear whether the dose of RIT in CLL patients should be adjusted according to cCD20 levels. The clinical efficacy of RIT in CLL, both as monotherapy and in combination with chemotherapeutic agents, was favorable. For 30 relapsed /refractory B−CLL patients, at the dose of 375 mg/m^2/d weekly, ×4, OR was 23%, and CR 0%, OR duration 20 weeks. And in the 34 patients untreated or treated with chlorambucil and prednisone, OR was 87%, CR 23%, OR duration 75 weeks[11].

Anti−CD52 (Campath−1H, alemtuzumab, ALT)

Alemtuzumab (ALT, Campath−1H) is a humanized anti− CD52 MoAb that binds to the cell membrane of virtually all normal cells as well as malignant lymphocytes whether B−cells or T−cells. However, stem cells do not express CD52 antigen [12,13]. This drug is a very pop topic for CLL therapy in recent years and got the approval of FDA in May 2001. It is studied widely, either used alone for CLL untreated or resistant to alkylating agents and FA, or combined with other drugs such as FA and Rituximab[14~23].

Alemtuzumab has been demonstrated significant activity in patients with previously untreated, relapsed, or refractory chronic lymphocytic leukemia (CLL), as well as in patients with T−cell prolymphocytic leukemia [24]. And Alemtuzumab (ALT, Campath−1H) is currently approved for the treatment of B−CLL resistant to alkylating agents and FA and also has been incorporated in novel conditioning regimens designed to facilitate stem cell transplantation in haematological malignancies. Most of the reported results present the use of ALT in heavily pretreated patients with B−CLL resistant to alkylating agents and/or FA. In 1997, Osterborg *et al.*[25] reported the results of treatment with ALT in 29 patients with relapsed or refractory CLL, 42% of the patients got major responses including 4% CR and 38% PR. The median duration response was 12 months. What´s worth

noting was that in 36% of patients CR was obtained in the bone marrow and in 32% splenomegaly completely resolved. In contrast, lymph nodes were less affected by ALT and lymphadenopathy was normalized in only 2 patients. More recent reports confirmed significant responses to ALT in CLL patients. Keating *et al.*[26] investigated the efficacy and safety of ALT in patients with relapsed or refractory B−CLL exposed to alkylating agents and having failed FA therapy. The overall response rate was 33% including 2% CR and 31% PR. The overall median time to response was 1.5 months and the median response duration was 8.7 months. The median survival for all patients was 16 months and 32 months for responders. In studies of Frampton JE *et al*[27] in patients with previously untreated B−CLL, subcutaneous (SC) administration of Alemtuzumab alone, or IV in combination with fludarabine, was highly effective, achieving OR rates of around 90%. IV alemtuzumab was also active in patients with chemotherapy−resistant/relapsed T−cell prolymphocytic leukaemia, with reported OR rates of 24%~76%.

The most notable success to data has been achieved with ALT, both in previously treated and untreated patients with CLL. In the vast majority of CLL patients ALT causes constant reduction of abnormal blood lymphocytes, usually in less than 4 weeks, and disappearance of CD5/CD19 co−expression cells from blood. However, this MoAb is not curative, because all patients eventually relapsed. Consequently, treatment with ALT may need to be associated with stem cell transplantation to consolidate and maintain long−term remissions[28]. More recently Albitar M. *et al.* reported that CD52 may be shed from cells and, once soluble, may bind to injected alemtuzumab, forming immune complexes, which demonstrated that soluble CD52 was detectable and useful in the staging and monitoring of patients with CLL, but also showed that soluble CD52 formed immune complexes with alemtuzumab and may influence the efficacy and toxicity of alemtuzumab therapy[29].

Alemtuzumab can also be used in BMT for depletion of normal T and B lymphocyte of both the recipient and donor for prevention of graft rejection and GVHD. It allows good stem cell recovery with resultant rapid engraftment and has a low risk of EBV−triggered secondary malignancy and does not interfere with blood stem cell mobilization. As a method of eliminating the malignant clone in B−CLL, alemtuzumab has shown remarkable efficacy in heavily pre−treated patients, a number of whom have progressed to autologous or allogeneic transplantation. The combination of tumour−depleting and immunosuppressive properties of alemtuzumab forwards the hope of providing improved treatment options for elderly patients with advanced B−CLL or indolent lymphoma whose prognosis is too poor currently with traditional regimens of high−dose myeloablative chemotherapy[30].

Over the past 5 years, a number of trials have demonstrated that alemtuzumab has clinical activity in

Table 1 Unlabeled and Labeled Antibodies Used to Treat Leukemia/Lymphoma

Antibodies	Antigen	Origin	Disease
Unlabeled			
Rituximab	CD20	chimeric	NHL, CLL
Campath−^{1}H	CD52	humanized	CLL, PLL
MEDI−507	CD2	humanized	ATL, NHL, GVHD
Allomune	CD2	humanized	NHL
HuM195(CMA−676)	CD33	humanized	AML, MDS, APL
LymphoCIDE	CD22	humanized	NHL
YTH12.5	CD3	humanized	T cell lymphoma
Drugs labeled			
Gemtuzumab ozogamicin	CD33	humanized	AML
BL22	CD22	murine	HCL
CMC−544	CD22	murine	NHL
Anti−B4−ricin	CD19	murine	NHL/ALL
HD37−dgRTA	CD19	murine	NHL/ALL
LMB−2	CD25	murine	HCL
Anti−CD7−PAP/ricin	CD7	murine	T−ALL/NHL
Gelonin−Anti−JL1	JI1	murine	AL
Isotopes labeled			
Bexxar	CD20	murine	NHL
Zevalin	CD20	murine	NHL
^{131}I−M195	CD33	murine	AML, MDS
^{131}I−HuM195	CD33	humanized	AML, MDS
^{90}Y−HuM195	CD33	humanized	AML, CML
^{213}Bi−HuM195	CD33	humanized	AML, CML
^{131}I−BC8	CD45	murine	ALL/ lymphoma
^{90}Y−BC8	CD45	murine	ALL/ lymphoma
^{188}Re−BW 250/183	CD66	murine	ricinAML, CML, ALL
^{90}Y−anti−Tac	CD25	murine	ATL
^{90}Y−anti−CD19	CD19	murine	NHL

T−cell leukemia and lymphoma such as T−ALL and cutaneous T−cell lymphoma (CTCL). For T−ALL, the objective response rate was 51%, with a 39.5% complete response (CR) rate[31~32].

Anti−CD33 (HuM195, CMA−676)

HuM195 is a humanized, unconjugated, anti−CD33 monoclonal antibody. The CD33 antigen is expressed on the surface of normal mature and immature myeloid cells, including colony−forming progenitor cells, and on leukemic blasts from 90% of patients with acute myeloid leukemia (AML). CD33 is not expressed by the normal stem cells, suggesting that in vivo ablation of CD33−bearing normal and leukemic myeloid cells might lead to the establishment of normal hematopoiesis by the remaining normal stem cells. But unconjugated anti−CD33 has limited effective [33]. In the study of Feldman E *et al.* 50 adult patients with relapsed or refractory AML were randomized to receive HuM195 at a dose of 12 or 36 mg/m^2 by intravenous infusion on days 1~4 and 15~18. Patients with stable or responding disease received two additional cycles on days 29~32 and 43~46. Of 49 evaluable patients, only two complete remissions and one partial remission were observed. All three responses were in patients treated at the 12mg/m^2 dose level and all had baseline blast percentages less than 30%. Decreases in blast counts ranging from 30 to 74% were seen in nine additional patients. This demonstrates that HuM195 as a single agent has observable, but minimal, anti−leukemic activity in patients with relapsed or refractory AML and activity is confined to patients with low burden disease[34].

Anti−CD2 (MEDI−507)

MEDI−507 is a humanized monoclonal antibody directed against CD2. Using an adult T−cell leukemia (ATL) xenograft model, Zhang Z *et al.* observed mice treated with MEDI−507 survived longer than the controls. Furthermore, prolonged treatment (6 months) of ATL with MEDI−507 significantly improved the outcome when compared with a short course (4 weeks) of therapy. Treatment with weekly MEDI−507 for 6 months led to a prolonged survival of the ATL−bearing mice that was almost comparable with the survival of the control group of mice that did not receive a tumor or therapeutic agent. Their results demonstrate that MEDI−507 has therapeutic efficacy on ATL in vivo and provides support for a clinical trial involving this monoclonal antibody in the treatment of patients with CD2−expressing leukemia and lymphoma[31].

Antibody−drug Conjugates

Traditional chemotherapy for acute leukemia often causes life−threatening toxic effects due to a lack of specificity for hematopoietic cells. Monoclonal antibodies and fusion proteins that target cell surface antigens on leukemic blasts are being evaluated for their cytotoxic effects and as a means of delivering chemotherapeutic agents directly to malignant cells. It is hoped that this strategy might selectively ablate malignant cells without many of the toxic effects commonly associated with conventional chemotherapy. Significant advances in the development of monoclonal antibodies conjugated to potent toxins or cytotoxic agents (immunoconjugates) have enabled improved targeting of leukemic cells with acceptable toxicities. Gemtuzumab ozogamicin, a calicheamicin−conjugated anti−CD33 monoclonal antibody, has demonstrated substantial efficacy in patients with acute myeloid leukemia (AML). The immunoconjugate BL−22, comprised of an anti−CD22 monoclonal antibody fused to a fragment of pseudomonas exotoxin PE38, has produced high response rates in hairy cell leukemia. Many other immunoconjugates are under researching and indicate potential significance, such as CD19 (HD37−dgRTA), CD22(RFB4−dgRTA), CD25 (RFB4−dgRTA).

Anti−CD33 (gemtuzumab; Mylotarg)

Therapeutic trials using unmodified anti−CD33 antibodies have limited success. Initial in vivo studies with an unmodified murine anti−CD33 antibody in patients with AML demonstrated that the antibody quickly bound to leukemia cells and that the antigen−antibody complex rapidly internalized following cell binding. However, when administered to patients with overt leukemia, unmodified antibody resulted in only brief decreases in peripheral blast counts, not in sustained response[35]. Using gemtuzumab ozogamicin (Mylotarg(R),

GO), a humanized anti−CD33 antibody conjugated with calicheamicin, the effectiveness of in vivo ablation of $CD33^+$ cells in patients with AML was improved[36]. Gemtuzumab ozogamicin has demonstrated substantial efficacy in patients with acute myeloid leukemia (AML) and has induced remissions in patients with favorable−, intermediate−, and poor−risk cytogenetics [37]. GO has recently been approved by FDA for use of relapsed and refractory acute myelogenous leukemia patients. GO is also approved for relapsed disease in patients older than 60 years, and now is being evaluated in combination with chemotherapy, in the setting of hematopoietic stem cell transplant, and in high−risk myelodysplasia[38].

Anti−CD19

CD19 antigen expresses on more than 90% of B lineage lymphoblastic cells. Anti−CD19 is a murine monoclonal antibody and several toxins were conjugated with this antibody for B cell NHL/ALL therapy, such as PAP (pokeweed antiviral protein), ricin and genistein.

The conjugate of the tyrosine kinase inhibitor genistein and the monoclonal antibody targets the immunotoxin to CD19 positive blast cells. It showed favorable pharmakokinetics [39] and anti−tumor activity in children and adults with advanced relapsed/refractory B−lineage ALL expressing CD19 on more than 50% of the leukemic blast cells. No life−threatening toxicity was encountered. In 15 patients, two CR and one partial remission (PR) were achieved with a monotherapy[40].

The anti−CD19 antibody conjugated with the pokeweed antiviral protein (PAP) immunotoxin was tested in a phase I trial. Two cycles of anti−CD19−PAP were administered parallel to a standard four−drug reinduction regimen in relapsed childhood ALL. Ten out of 15 patients achieved CR and two PR [41]. This study exemplifies that combined chemotherapy and antibody treatment may result in effective regimens for relapsed/refractory ALL.

Anti−B4−blocked ricin is an immunotoxin consisting of anti−B4 murine monoclonal antibody (anti−CD19) and "blocked ricin" toxin. Many studies demonstrated it had definitely effect in B cell ALL and NHL. In a research of a phase II study of adjuvant therapy with anti−B4−blocked ricin after autologous bone marrow transplantation for patients with relapsed B−cell non−Hodgkin´s lymphoma, a total of 83 courses of Anti−B4−ricin were administered, with 31 patients receiving two or more courses of therapy. The 4−year disease−free survival and overall survival are estimated at 56% and 72%, respectively. Twenty−six patients remain in CR after a median follow−up of 54.5 months. Reversible toxicities included hepatic transaminase elevations, thrombocytopenia, myalgias, fatigue, nausea, hypoalbuminemia, and dyspnea. Human antimouse antibody (HAMA) and/or human antiricin antibody (HARA) responses occurred in 23 patients at a median of 22 days from the initiation of Anti−B4−bR therapy. This study demonstrates that Anti−B4−bR can be administered safely to patients as adjuvant therapy early after ABMT for B−cell NHL. The toxicities are tolerable and reversible [42]. In the Cancer and Leukemia Group B Study 9311, after the first remission, Forty−six CD19 positive ALL patients received the anti−B4−blocked ricin, 80% were able to receive both courses. Molecular monitoring before and after the experimental course of intensification did not show a consistent change in the number of leukemia cells remaining. The most common toxicity was asymptomatic transient elevation of liver function tests in 72% of patients. Lymphopenia occurred in 46% of patients. Two patients developed antibodies to the anti−B4−blocked ricin. That showed intensification therapy with anti−B4−blocked ricin is feasible for patients with CD19 positive ALL[43]. But anti−B4−blocked ricin had limited activity in CLL [44], maybe because anti−CD19 mAb couldn´t be taken up by B CLL cell[45].

HD37−dgRTA is an anti−CD19 monoclonal antibody conjugated to a deglycosylated ricin A chain (dgRTA). Herrera L *et al.* demonstate that it can kill B−lineage non−Hodgkin´s lymphoma (NHL) cells in vitro, in vivo and in adult patients with B−lineage ALL [46]. And in vitro it can selectively kill pediatric precursor B−lineage leukemia cells. But it should be further evaluated for the therapy of pediatric B−lineage ALL[47].

Anti− CD22 and anti− CD25

Besides anti−CD20, anti−CD52 and anti−CD19, clinical experience with other MoAbs in patients with

chronic lymphoid leukemias is very limited. However preliminary results with MoAbs combined with toxins and directed against CD22 and CD25 antigens seem to be promising, especially in the treatment of HCL [48~49]. CD22 antigen, expressing RFB4 epitope, is an adhesion molecule expressed exclusively on B cells. Clasic or variant hairy cells are virtually always strongly positive for CD22[50]. In contrast CD22 is not present on the cell surface in the early stages of B-cell development and is not expressed on stem cells. In the recent years recombinant immunotoxin, RFB4 (dsFv)-PE-38 (BL22), that contains the variable domain (Fv) of the anti- CD22 monoclonal antibody RFB4, was designed to target CD22 expressing cells[51~52]. Recombinant immunotoxins are fusion proteins composed of the Fv domains of antibodies fused to bacterial or plant toxins that are being developed for the targeted therapy of neoplastic diseases. In BL22 immunotoxin the Fv fragment in a single-chain form is fused to a truncated form of Pseudomonas exotoxin A called PE-38[53]. And anti-CD22 (RFB4-dgRTA) immunotoxins (ITs) is a murine IgG(1) monoclonal antibody (Mabs) conjugated to a deglycosylated ricin A chain (dgRTA). It is effective in killing B-lineage non-Hodgkin´s lymphoma (NHL) cells in vitro, in vivo and in adult patients with B-lineage NHL. But the potential of these agents for the treatment of childhood B-precursor acute lymphoblastic leukemia (ALL) is unknown [54]. Another immunoconjugate of CD22 (CMC-544) is a combination of N-acetyl-gamma-calicheamicin dimethyl hydrazide (CalichDMH), a potent DNA-binding cytotoxic antitumor antibiotic. The results of the CMC-544 preclinical research showed it exerted potent cytotoxicity against $CD22^+$ B-cell lymphoma (BCL) cell lines. CMC-544 caused a potent inhibition of growth of small but established BCL xenografts leading to cures. CMC-544 prevented the establishment of BCL xenografts and also caused regression of large BCLs[55].

The malignant cells in 80% of HCL cases express high surface levels of the subunit-a of the IL-2 receptor (IL-206 T. ROBAK 2Ra). This receptor is also known as CD25 or p55[56]. A recombinant single-chain immunotoxin composed of the variable domains of the anti-Tac (CD25) monoclonal antibody fused to a truncated form of Pseudomonas exotoxin is particularly active against $CD25^+$ HCL cells [57]. This anti-Tac (Fv)-PE-38 (LMB-2) MoAb was very cytotoxic in vivo in all HCL patients with IC50 as low as 0.5 ng/ml. Malignant cells freshly obtained from patients with CLL were also sensitive to LMB-2 but less sensitive than HCL cells[58].

Anti-CD7

CD7 antigen is a surface molecule that expresses on 99% of T-lineage lymphoblastic cells. Anti-CD7 is murine monoclonal antibody, and it was under researching to be conjugated with ricin, or PAP (pokeweed antiviral protein) for T-ALL therapy. In fact, T-lineage ALL antibody therapy has been far less intensively explored, which may be due to the fact that immunotherapy was first developed for treatment of B-lineage NHL as a more frequent disease. T-cell antibodies were often explored for T-cell depletion in the setting of stem cell transplantation, such as anti-CD52. Several studies showed anti-CD7-ricin [59] and anti-CD7-PAP [60] had activity in mouse models for T-cell leukemias, but limited clinical experience is available only for these antibodies. Anti-CD7-ricin was tested in a phase I trial in patients with relapsed T-cell lymphoma (>30% CD7 positive blast cells). Two PRs were achieved in 11 patients [61]. No new development was reported more recent years.

Anti-JL1 mAb

JL1 is a unique thymocyte-specific surface molecule, which was detected with the monoclonal antibody (mAb), anti-JL1. Interestingly, JL1 was shown to be expressed in most leukemias, irrespective of their immunophenotype, and subpopulations of normal bone marrow (BM) mononuclear cells (MNCs). Shin YK et al. demonstrated that the proliferation of cultured human leukemia cells was dramatically inhibited in vitro by anti-JL1 mAb conjugated with the polypeptide toxin, gelonin, but not by gelonin alone. They also investigated the reactivity of the anti-JL1 mAb against normal human tissues to evaluate possible side effects. The results showed the cytotoxic effects of anti-JL1-based immunotoxin against JL1-positive leukemic cells, sparing most normal tissues other than thymocytes and

some BM MNCs. Gelonin-conjugated anti-JL1 mAb immunotoxin may be developed as a potential immunotherapeutic agent in the treatment of various types of JL1-positive acute leukemias[62].

Radiolabaled monoclonal antibody

There are two kinds of radionuclides used for immunoradiotherapy: isotopes emitting alpha particles and isotopes emitting beta particles. Alpha particles have short ranges and high energy and it can selectively kill the tumor cells targeted by the mAb, without nonspecific toxicity. Monoclonal antibodies conjugated with alpha particles are suited for the treatment of low-volume tumor and residual disease. Beta particles have long ranges and relatively low energy, and it can deliver a substantial radioation dose to the bone marrow, but it also increase nonspecific toxicity and produces prolonged myelosuppression requiring high dose hematopoietic stem cell transplantation. Monoclonal combined with beta particles are usually used for high burden tumor and BMT preparative regimens. The beta emitters studied most commonly for immunoradiotherapy of malignant hematological diseases are ^{90}Y and ^{131}I. ^{213}Bi and ^{225}Ac are alpha emitters.

Compared with other β-particle emitters, ^{131}I has several disadvantages. First, because of long-ranged γ-emissions, patients must be hospitalized and isolated. Second, the long physical half-life of ^{131}I (8.1 days) delays the time from treatment to stem cell infusion in patients undergoing transplantation. Third, when IgG is labeled with high doses of ^{131}I, the ability of the antibody to bind to the target antigen is dramatically reduced. However ^{90}Y offers several advantages over ^{131}I for myeloablation. After internalization of antigen-antibody complexes into target cells, ^{90}Y are better retained within these cells. Furthermore, because ^{90}Y is a pure β-emitter, large doses can be given safely in the outpatient setting with fewer consequences for medical personnel or patients´ families[63](Table 2).

Anti-CD20 (Bexxar and Zevalin)

Treatment of lymphoma with unconjugated anti-CD20 mAb (Rituximab) has shown efficacy in clinical trials and have gained approval from the Food and Drug Administration (FDA). Although Rituximab produces responses in approximately 50% of cases of relapsed, low grade NHL. Most of these responses are partial remissions, and cure remains elusive. However, the use of radionuclides to exploit the specific targeting properties has been a major development in mAb therapeutics. Two radionuclide-bearing mAbs have recently been approved by the FDA: in February 2002, ^{90}Y-labeled anti-CD20 mAb (Zevalin) became the first radioimmunoconjugate to be approved by the FDA for the treatment of cancer. ^{131}I-labeled anti-CD20 mAb (Bexxar) was approved in June 2003[64].

Zevalin is a murine anti-CD20 antibody combined with ^{90}Y and was approved in 2002 for the treatment of patients with relapsed or refractory low-grade follicular or transformed B-cell NHL, including patients with follicular lymphoma refractory to rituximab. Yttrium-90 provides advantages over iodine-131 because it delivers higher beta energy [65]. Zevalin has a higher overall response rate (OR) than rituximab, as demonstrated in two separate clinical trials. The first trial randomized 143 rituximab-naive patients with relapsed NHL to receive rituximab or Zevalin. The OR for Zevalin was 80% compared with 56% for rituximab. The second trial tested the efficacy of Zevalin in patients who were rituximab-refractory; the OR was 74%. The main tox-

Table 2 Characteristics of several isotopes used for hematological radioimmunotherapy

Isotopes	Particles emitted	Half-life	Particle energy (Mev)	Mean range (mm)
Iodine-131	β, γ	8.0d	970	0.8
Rhenium-188	β, γ	17h	2120	2.4
Yttrium-90	β	64h	2280	2.7
Bismuth-213	α	46min	5982	0.05-0.08
Actinium-225	α	10d	5935	0.05-0.08

icity of Zevalin was reversible myelosuppression. These indicate that Zevalin can produce a higher OR than rituximab, and single-dose Zevalin is another treatment alternative for patients with relapsed low grade NHL. It is well tolerated even by older adults. The exact role of Zevalin in the therapy of NHL is undetermined. New studies are underway to explore whether patients can safely receive a second dose of Zevalin and to combine Zevalin with high-dose chemotherapy and stem cell rescue[66].

Bexxar [B1, Iodine-131 tositumomab, iodine-131 anti-B1 antibody] is also a murine antibody conjugated to iodine 131 that recognizes and binds to the CD20 (B1) antigen which is found specifically on B lymphocytes. ^{131}I emits both therapeutic beta radiation and highly penetrating gamma emissions. The lower energy of the beta particles emitted by ^{131}I is used to treat tumors. The gamma radiation allows both dosimetry and biodistribution studies to be performed. Patients who receive bexxar therapy are usually hospitalized in radioprotection wards, and are treated by specially trained hospital staff [67]. In clinical trials of Bexxar, objective response rates ranged from 54%~71% in heavily pretreated patients. In 76 newly diagnosed patients, the objective response rate was 97%, and 63% of patients achieved complete responses[68].

Anti-CD20 was also conjugated with ^{213}Bi for the treatment of B-cell CLL, and in vitro it killed B cells more effectively than equivalent doses of external gamma irradiation[69]. No in vivo experiment reported.

Anti-CD33 (labeled with ^{131}I, ^{90}Y, ^{225}Ac or ^{213}Bi)

Besides conjugated with calicheamicin, CD33 was also conjugated with some radioisotope, either emitting beta particles or alpha particles. Radioimmunotherapy with beta (beta) particle-emitting isotopes (131I or 90Y) has produced significant responses while minimizing radiation exposure to normal tissues in both nonmyeloablative and myeloablative regimens. When labeled with the beta-emitters ^{131}I and ^{90}Y, CD33 (HuM195) can eliminate large leukemic burdens in patients, but it produces prolonged myelosuppression requiring hematopoietic stem cell transplantation at high doses. Unlike beta particle-emitting isotopes, alpha emitters can selectively kill individual cancer cells with a single atomic decay. To enhance the potency of native HuM195 yet avoid the nonspecific cytotoxicity of beta-emitting constructs, the alpha-emitting isotope ^{213}Bi was conjugated to HuM195. Targeted alpha (alpha) particle therapy with ^{213}Bi-labeled HuM195 offers the possibility of more selective tumor cell kill [70]. An institute, called Memorial Sloan-Kettering Cancer Center in New York, began to research on the radioimmunotherapy for acute leukemia since 1991. They published many papers and in 2002 the results was declared in ASH meeting. At first, they labeled M195 (mouse antibody) with iodine-131, the data of the phase I clinical trial showed that iodine-131 labeled CD33 had definite effect on myeloid leukemia therapy, however, 37% of the patients developed human anti-mouse antibody, preventing retreatment. Then the humanized CD33 (HuM195) was adopted, and results showed specific bone marrow targeting without an immunogenic response. But iodine-131, which emits β particles, produced prolonged myelosuppression requiring hematopoietic stem cell transplantation at high doses. To avoid nonspecific cytotoxicity of beta-emitting, the α-emitting isotope ^{213}Bi was conjugated to HuM195. Eighteen patients with relapsed and refractory acute myelogenous leukemia or chronic myelomonocytic leukemia were treated with ^{213}Bi-HuM195. No significant extramedullary toxicity was seen. All 17 evaluable patients developed myelosuppression, with a median time to recovery of 22 days. Nearly all the ^{213}Bi-HuM195 rapidly localized to and was retained in areas of leukemic involvement, including the bone marrow, liver, and spleen. Absorbed dose ratios between these sites and the whole body were 1000-fold greater than those seen with β-emitting constructs in this antigen system and patient population. Fourteen (93%) of 15 evaluable patients had reductions in circulating blasts, and 14 (78%) of 18 patients had reductions in the percentage of bone marrow blasts. This study demonstrated the safety, feasibility, and antileukemic effects of ^{213}Bi-HuM195(2002, ASH meeting). The short path length of this alpha-emitter could theoretically allow killing of the targeted leukemic cell without damage to normal neighbors and are better suited for the treatment of

low−volume or residual disease. Now ^{213}Bi−HuM195 is undergoing the phase II clinical trial. Recently (2003) they also studied ^{131}I−labeled HuM195 combined with busulfan and cyclophosphamide (BuCy) as conditioning for allogeneic BMT in advanced myeloid leukemia, the data confirmed the feasibility of adding ^{131}I −labeled CD33 to a standard BMT preparative regimen [71~80].

^{90}Y−HuM195 is also undergoing trials. In a phase I trial at MSKCC, ^{90}Y−HuM195 was studied in patients with relapsed or refractory AML. Nineteen patients were treated with escalating doses of ^{90}Y−HuM195 (0.1 to 0.3 mCi/kg). Myelosuppression lasted 9 to 62 days, and the maximum tolerated dose without stem cell rescue was 0.275 mCi/kg. Thirteen patients had reductions in bone marrow blasts, and 1 patient achieved a complete remission lasting 5 months. All patients treated with 0.3 mCi/kg had hypocellular bone marrow biopsies performed 2 or 4 weeks after treatment, without evidence of leukemia. These results suggest that ^{90}Y−HuM195 will be useful as conditioning before stem cell transplantation[81]. Clinical trials investigating this agent as part of preparative regimens for autologous and nonmyeloablative allogeneic stem cell transplantation are now underway.

More recently, ^{225}Ac has been conjugated to HuM195. ^{225}Ac has a 10−day half−life and decays by α emission through three atoms, each of which also emits a α−particle. In vitro, ^{225}Ac coupled to internalizing Mabs specifically killed leukemia, lymphoma, breast, ovarian, neuroblastoma, and prostate cancer cells at doses 1,000 times less than ^{213}Bi−containing radioimmunoconjugates. In xenograft models of disseminated human lymphoma, single doses at nanocurie levels of tumor−specific constructs prolonged survival and cured a substantial fraction of animals without toxicity. A phase I trial of ^{225}Ac−HuM195 in advanced myeloid leukemias is planned[82].

Anti−CD45 (^{131}I labeled, ^{90}Y labeled)

The vast majority of leukemias and normal stem cells express the cell surface antigen CD45, so radiolabeled anti−CD45 (BC8) allows the delivery of myeloablative radiation to bone marrow, spleen and common sites of leukemic involvement. Consequently, ^{131}I−labeled anti−CD45 antibody has been combined with traditional preparative regimens for patients receiving bone marrow transplantation for acute leukemia [83]. When combined with conventional CY/TBI, ^{131}I−anti−CD45 can safely deliver substantial supplemental doses of radiation to bone marrow (approximately 24 Gy) and spleen (approximately 50 Gy) [84].

Another study was undertaken by Vallera DA *et al.* to investigate the suitability of using anti−CD45 monoclonal antibody for delivering the high−energy beta−particle emitting isotope ^{90}Y to lymphohematopoietic target cells *in vivo*. The antibody recognized the CD45 antigen expressed on the surface of both normal and malignant hematopoietic cells and the results showed that it reacted with both CD45−expressing normal peripheral blood cells and leukemia cells from patients. The antibody was readily labeled with ^{90}Y using the highly stable chelate 1B4M−DTPA and the radioimmunoconjugate was designated ^{90}Y−anti−CD45. The agent selectively bound to CD45 (+) B cell line Daudi, but not CD45(−) control cells. These mice treated with ^{90}Y −anti−CD45 displayed a significantly better anti−tumor effect than a control antibody labeled with ^{90}Y and survived over 135 days with no evidence of tumor. Because radiolabeled anti−CD45 antibody can be used to deliver radiation selectively to lymphohematopoietic tissue, these data indicate that this agent may be used to improve treatment of lymphocytic leukemia and lymphoma, when combined with hematopoietic stem cell transplantation in the future[85].

Anti−CD25 (^{90}Y labeled)

CD25 (IL−2R alpha) is an ideal choice for a target antigen as it is over−expressed by a number of tumor cells. Anti−Tac is a murine MAb that binds to IL−2R. ^{90}Y labeled anti−Tac (which targets the interleukin−2 receptor−alpha [IL−2R alpha]) provide a useful approach for treatment of adult T−cell leukemia (ATL). In a phase I/II trial, 18 patients with adult T−cell leukemia were treated with ^{90}Y−anti−Tac. Nine patients were treated in a phase I dose escalation trial (5 to 15 mCi), and the remaining 9 patients were treated with a uniform dose of 10 mCi. Patients who had a remission were eligible for additional cycles of treatment. Of 16

evaluable patients, 7 had partial remissions (mean duration, 9.2 months), and 2 had complete remissions. Six patients developed human antimouse antibodies[86].

Anti-CD19 (^{90}Y labeled)

In studies performed in the B cell lymphoma murine model, anti-tumor activity of ^{90}Y labeled Anti-CD19 was dose dependent and the best results were observed in mice receiving a single dose of approximately 300 uCi. The anti-CD19 antibody had significantly better anti-tumor activity as compared to a control ^{90}Y-labeled antibody and most mice survived over 119 days with no evidence of tumor ($p<0.003$). Histology studies showed no significant injury to the kidney, liver, or small intestine. Because radiolabeled anti-CD19 antibody can be used to deliver radiation selectively to lymphohematopoietic tissue, these data support the use of ^{90}Y anti-CD19 antibodies in treating B-cell malignancies [87]. D Ma *et al.* compared the efficacy of the ^{90}Y-labeled anti-CD19 to reduce lymphoma in a nude mouse xenograft solid tumor model, after measurable lymphoma appeared. Reduction in tumor size began at day 3 in ^{90}Y-treated group, but tumor began to recur in many animals 9 days after the treatments. In contrast, the tumor in the control group showed no regression. There was a significant prolongation of median survival time from xenograft in the ^{90}Y-labeled antibody construct-treated group in comparison to the control group. Specificity of the radioimmunotherapy was also shown. At the same time, the results showed ^{90}Y labeled anti-CD19 antibody has efficacy comparable to ^{90}Y-labeled anti-CD20 antibody (Zevalin) in the treatment of mice bearing human lymphoma xenografts. These data suggest that CD19-targeted RIT merits further study[88].

Anti-CD66c (^{188}Re labeled)

CD66c known as nonspecific cross-reacting antigen (NCA), is a glycoprotein expressed on myeloid cells but not on leukemia cells. BW 250/183 is a murine monoclonal IgG1 antibody directed at CD66c [89]. ^{188}Re is a radiometal with a 17-hour half-life. It emits both β and γ radiation. A phase I dosimetry trial showed that administration of ^{188}Re-BW 250/183 resulted in a favorable biodistribution in 11 of 12 patients, with significant amounts of radiation delivered to the marrow[90]. In a subsequent trial, 36 patients with high-risk AML or myelodysplastic syndrome were treated with ^{188}Re-BW 250/183 prior to hematopoietic cell transplantation. After treatment with radiolabeled antibody, patients received one of three preparative regimens: total body irradiation (12 Gy) plus cyclophosphamide (120 mg/kg), busulfan (12.8 mg/kg) plus cyclophosphamide (120 mg/kg), or total body irradiation (12 Gy) plus thiotepa (10 mg/kg) and cyclophosphamide (120 mg/kg). Thirty-one patients received allogeneic grafts (mostly T-cell-depleted), 1 received a syngeneic graft, and 4 received autologous grafts. Favorable biodistribution of ^{188}Re-BW 250/183 occurred in all patients. Besides the toxicity normally associated with the conventional preparative regimens, no additional toxicity attributable to the radiolabeled antibody occurred. Engraftment occurred in all patients and was not delayed. Disease-free survival was 45% at the median follow-up of 18 months. Nine of 35 evaluable patients relapsed. This study suggests that ^{188}Re-BW 250/183, similar to ^{90}Y-HuM195 and ^{131}I-BC8, may deliver significant doses of radiation to the marrow without excessive toxicity[91].

CONCLUSION

Over the past decade the potential for delivering targeted therapy against malignant disease by the use of monoclonal antibodies (MAbs) has begun to be realized. Hematological malignancies, because of the relative accessibility of the malignant cell in blood and bone marrow and the understanding of hemopoietic lineage-specific antigens, have provided a successful testing ground for this therapy. There have been many technical developments that have allowed the safe delivery of more potent antibody constructs. The development of human or chimeric antibodies has largely overcome the problems associated with host immune responses to murine-derived MAbs. Protein engineering to combine MAbs with radioisotopes and toxins has made available a new range of agents with clinical activity[92]. This review gave most of all therapeutic antibodies for malignant hematological diseases. The trials of the next decade will explore issues such as: whether these drugs can improve survival; the optimal strategies and timing for clinical

use; whether increasing potency of MAbs (as in immunoconjugates) will increase toxicity and, finally, what other potential molecules may be targeted or other toxins and isotopes can be conjugated.

REFERENCES

1. Mellstedt H. Monoclonal antibodies in human cancer. Drugs Today (Barc), 2003, 39 Suppl C:1−16.
2. McLaughlin P, Cabanillas F, Grillo−Lopez AJ, *et al.* IDEC−C2B8 anti−CD20 antibody: final report on a Phase III pivotal trials in patients with relapsed low−grade follicular lymphoma. Blood, 1996, 88: 90a.
3. Coiffier B, Haioun C, Ketterer N, *et al.* Rituximab (anti CD20 monoclonal antibody) for the treatment of patients with relapsing or refractory aggressive lymphoma: a multicenter phase II study. Blood, 1998, 92: 1927−1932.
4. O´Brien S, Freireich E, Andreeff M, *et al.* Phase I/II study of Rituxan in chronic lymphocytic leukemia. Blood, 1998, 92: 105.
5. Byrd JC, White CA, Link B, *et al.* Rituximab therapy in previously treated Waldenstrom´s macroglobulinemia: preliminary evidence of activity. Blood, 1998, 92: 106.
6. Milpied N, Basseur B, Antoine C, *et al.* Chimeric anti−CD20 monoclonal antibody (Rituximab) in B post transplant lymphoproliferative disorders (BPTLDs): a retrospective analysis on 32 patients. Blood, 1999, 94: 631.
7. Demidem A, Lam T and Alas S *et al.* Chimeric anti−CD20 (IDEC−C2B8) monoclonal antibody sensitizes a B cell lymphoma cell line to cell killing by cytotoxic drugs. Cancer Biotherapy and Radiopharmacsuticals, 1997, 12: 177−186.
8. Di Gaetano N, Xiao Y and Erba E, *et al.* Synergism between fludarabine and rituximab revealed in a follicular lymphoma cell line ressistant to the cytotoxic activity of either drug alone. Br J Haemat, 2001, 114: 800−809.
9. Nabhan C and Rosen S T. Conceptual aspects of combining rituximab and Campath−1H in the treatment of chronic lymphocytic leukaemia. Seminars in Oncology, 2002, 29: 75−80.
10. Manshouri T, Do K A, Wang X, *et al.* Circulating CD20 is detectable in the plasma of patients with chronic lym phocytic leukemia and its prognostic significance. Blood, 2003, 101: 2507−2513.
11. Robak T. Monoclonal antibodies in the treatment of chronic lymphoid leukemias. Leuk Lymphoma, 2004, 45: 205−19.
12. Treumann A, Lifely M R, Schneider P, *et al.* Primary structure of CD52. J Bio Chem, 1995, 270: 6088−6099.
13. Ginaldi L, De Martinis M, Matutes E. Levels of expression of CD52 in normal and leukemic B and T cells: cor relation with in vivo therapeutic responses to Campath−1H. Leuk Res, 1998 Feb,22: 185−191.
14. Lundin J, Porwit−MacDonald A, Rossmann ED, *et al.* Cellular immune reconstitution after subcutaneous alemtuzumab (anti−CD52 monoclonal antibody, CAMPATH−^{1}H) treatment as first−line therapy for B−cell chronic lymphocytic leukaemia. Leukemia, 2004 Mar, 18: 484−490.
15. Rieger K, Von Grunhagen U, Fietz T, *et al.* Efficacy and tolerability of alemtuzumab (CAMPATH−^{1}H) in the salvage treatment of B−cell chronic lymphocytic leukemia−change of regimen needed? Leuk Lymphoma, 2004 Feb, 45: 345−349.
16. Lozanski G, Heerema NA, Flinn IW, *et al.* Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. Blood, 2004 May 1, 103: 3278−3281.
17. Nabhan C, Dyer MJ, Rosen ST. Current status of monoclonal antibody therapy for chronic lymphocytic leukemia. Oncology (Huntingt), 2003 Feb,17: 253−262.
18. Faderl S, Thomas DA, O´Brien S, *et al.* Experience with alemtuzumab plus rituximab in patients with relapsed and refractory lymphoid malignancies. Blood, 2003 May 1,101: 3413−3415.
19. Osterborg A, Mellstedt H, Keating M. Clinical effects of alemtuzumab (Campath−^{1}H) in B−cell chronic lymphocytic leukemia. Med Oncol, 2002,19 Suppl: S21−6.
20. McCune SL, Gockerman JP, Moore JO, Alemtuzumab in relapsed or refractory chronic lymphocytic leukemia and prolymphocytic leukemia. Leuk Lymphoma, 2002 May, 43: 1007−11.
21. Lundin J, Kimby E, Bjorkholm M, Phase II trial of subcutaneous anti−CD52 monoclonal antibody alemtuzumab (Campath−^{1}H) as first−line treatment for patients with B−cell chronic lymphocytic leukemia (B−CLL). Blood, 2002 Aug 1, 100: 768−73.
22. Dumont FJ. CAMPATH (alemtuzumab) for the treatment of chronic lymphocytic leukemia and beyond. Expert Rev Anticancer Ther,2002 Feb, 2: 23−35.
23. Schulz H, Winkler U, Staak JO, *et al.* The Monoclonal Antibodies Campath−^{1}H and Rituximab in theTherapy of Chronic Lymphocytic Leukemia. Onkologie, 2000 Dec, 23: 526−532.
24. Moreton P, Hillmen P. Alemtuzumab therapy in B−cell lymphoproliferative disorders. Semin Oncol, 2003 Aug, 30: 493−501.
25. Osterborg A, Dyer M J, Bunjes D, *et al.* Phase II multicenter study of human CD52 antibody in previously treated chronic lymphocytic leukemia. Journal of Clinical Oncology, 1997, 15: 1567−1574
26. Keating M J, Flinn I and Jain V. *et al.* Therapeutic role of alemtuzumab (Campath−^{1}H) in patients who have failed fludarabine: result of a large international study, Blood, 2002, 99:

3554−3561

27. Frampton JE, Wagstaff AJ. Alemtuzumab. Drugs, 2003, 63: 1229−1243; discussion 1245−1246.

28. Robak T. Monoclonal antibodies in the treatment of chronic lymphoid leukemias. Leuk Lymphoma, 2004 Feb, 45: 205−19.

29. Albitar M, Do KA, Johnson MM, *et al.* Free circulating soluble CD52 as a tumor marker in chronic lymphocytic leukemia and its implication in therapy with anti−CD52 antibodies. Cancer, 2004 Sep 1,101: 999−1008.

30. Hale G, Slavin S, Goldman JM, Alemtuzumab (Campath−^{1}H) for treatment of lymphoid malignancies in the age of non-myeloablative conditioning? Bone Marrow Transplant, 2002 Dec, 30: 797−804.

31. Zhang Z, Zhang M and Ravetch JV, *et al.* Effective therapy for a murine model of adult T−cell leukemia with the humanized anti−CD2 monoclonal antibody, MEDI−507. Blood, 2003 Jul 1,102: 284−288.

32. Keating MJ, Cazin B, Coutre S, *et al.* Campath−^{1}H treatment of T−cell prolymphocytic leukemia in patients for whom at least one prior chemotherapy regimen has failed. J Clin Oncol,2002 Jan 1, 20(1):205−213.

33. Bernstein ID. CD33 as a Target for Selective Ablation of Acute Myeloid Leukemia. Clin Lymphoma, 2002 Mar, 2: S9−S11.

34. Feldman E, Kalaycio M and Weiner G, *et al.* Treatment of relapsed or refractory acute myeloid leukemia with humanized anti−CD33 monoclonal antibody HuM195. Leukemia, 2003 Feb,17: 314−318.

35. Appelbaum FR. Antibody−targeted therapy for myeloid leukemia. Semin Hematol, 1999 Oct, 36: 2−8.

36. Milenic DE. Monoclonal antibody−based therapy strategies: providing options for the cancer patient. Curr Pharm Des, 2002, 8: 1749−1764.

37. Tallman MS. Monoclonal antibody therapies in leukemias. Semin Hematol, 2002 Oct, 39: 12−19.

38. Tomblyn MR, Tallman MS. New developments in antibody therapy for acute myeloid leukemia. Semin Oncol, 2003, 30: 502−508.

39. Chen CL, Levine A, Rao A, *et al.* Clinical pharmacokinetics of the CD19 receptor−directed tyrosine kinase inhibitor B43−Genistein in patients with B−lineage lymphoid malignancies. J Clin Pharmacol, 1999, 39: 1248−1255.

40. Uckun FM, Nachman JB, Sather HN, *et al.* Poor treatment outcome of Philadelphia chromosome−positive pediatric acute lymphoblastic leukemia despite intensive chemotherapy. Leuk Lymphoma, 1999, 33: 101−106.

41. Seibel NL, Krailo M, ONeill K, Franklin J, Uckun F, Reaman GH Phase I study of B43−PAP immunotoxin in combination with standard 4−drug induction for patients with CD19^{+} acute lymphoblastic leukemia (ALL) in relapse. A Childrens Cancer Group Study. Blood, 1998, 92: 1651.

42. Grossbard ML, Multani PS, Freedman AS, *et al.* A Phase II study of adjuvant therapy with anti−B4−blocked ricin after autologous bone marrow transplantation for patients with relapsed B−cell non−Hodgkin´s lymphoma. Clin Cancer Res,1999, 5: 2392−2398.

43. Szatrowski TP, Dodge RK, Reynolds C, *et al.* Lineage specific treatment of adult patients with acute lymphoblastic leukemia in first remission with anti−B4−blocked ricin or high−dose cytarabine: Cancer and Leukemia Group B Study 9311. Cancer, 2003, 97: 1471−80.

44. Tsimberidou AM, Giles FJ, Kantarjian HM, *et al.* Anti−B4 blocked ricin post chemotherapy in patients with chronic lymphocytic leukemia−−long−term follow−up of a monoclonal antibody−based approach to residual disease. Leuk Lymphoma, 2003, 44: 1719−1725.

45. Sieber T, Schoeler D, Ringel F, *et al.* Selective internalization of monoclonal antibodies by B−cell chronic lymphocytic leukaemia cells. Br J Haematol, 2003, 121: 458−461.

46. Herrera L, Yarbrough S and Ghetie V, *et al.* Treatment of SCID/human B cell precursor ALL with anti−CD19 and anti−CD22 immunotoxins. Leukemia, 2003, 17: 334−338.

47. Herrera L, Farah RA and Pellegrini VA, *et al.* Immunotoxins against CD19 and CD22 are effective in killing precursor−B acute lymphoblastic leukemia cells in vitro. Leukemia, 2000, 14: 853−858.

48. Hale G. The CD52 antigen and development of the CAMPATH antibodies. Cytotherapy, 2001, 3: 137−143.

49. Kreitman R J, Wilson W H and Robbins D, *et al.* Responses in refractory hairy cell leukemia to a recombinant immunotoxin. Blood, 1999, 94: 3340−3348.

50. Robbins D H, Margulies I and Stetler−Stevenson M. Hairy cell leukemia, a B−cell neoplasm that is particularly sensitive to the cytotoxic effect of anti−Tac(Fv) PE−38 (LMB−2). Clinical Cancer Research, 2000, 6: 693−700.

51. Salvatore G, Beers R and argulies I. Improved cytotoxic activity toward cell lines and fresh leukemia cells of a mutant anti−CD22 immunotoxin obtained by antibody phage display. Clinical Cancer Research, 2002, 8: 995−1002.

52. Sausville E A, Headlee D, Stetler−Stevenson M, *et al.* Continuous infusion of the anti−CD22 immunotoxin IgG RFB4−SMPT−dgA in patients with B−cell lymphoma a phase I study. Blood, 1995, 85: 3457−3465.

53. Brinkmann U. Recombinant immunotoxins protein engineering for cancer therapy. Molecular Medicine Today, 1996, 2: 439−446.

54. Herrera L, Yarbrough S, Ghetie V, *et al.* Treatment of

SCID/human B cell precursor ALL with anti−CD19 and anti−CD22 immunotoxins. Leukemia, 2003 Feb,17: 334−338.

55. DiJoseph JF, Armellino DC, Boghaert ER, *et al.* Antibody−targeted chemotherapy with CMC−544: a CD22−targeted immunoconjugate of calicheamicin for the treatment of B−lymphoid malignancies. Blood, 2004 Mar 1,103: 1807−1814.

56. Robbins B A, Ellison D J, Spinosa J C, *et al.* Diagnostic application of two color cytometry in 161 cases of hairy cell leukaemia, Blood, 1993, 82: 1277−1287.

57. Kreitman R J. Immunological treatment of hairy −cell leukaemia. Best Practice and Research Clinical Haematology, 2003, 16: 117−133.

58. Vallera DA, Burns LJ, Frankel AE, *et al.* Laboratory preparation of a deglycosylated ricin toxin A chain containing immunotoxin directed against a CD7 T lineage differentiation antigen for phase I human clinical studies involving T−cell malignancies. J Immunol Methods,1996, 197:69−83.

59. Waurzyniak BJ, Schneider EA, Tumer N, *et al.* In vivo toxicity, pharmacokinetics, and antileukemic activity of TXU (anti −CD7) −pokeweed antiviral protein immunotoxin. Clin Cancer Res, 1997, 3: 881−890

60. Frankel AE, Laver JH, Willingham MC, *et al.* Therapy of patients with T−cell lymphomas and leukemias using an anti−CD7 monoclonal antibody−ricin A chain immunotoxin. Leuk Lymphoma, 1997, 26: 287−298.

61. Robbins D H, Margulies I and Stetler−Stevenson M, *et al.* Hairy cell leukemia, a B−cell neoplasm that is particularly sensitive to the cytotoxic effect of anti−Tac (Fv) PE−38 (LMB−2), Clinical Cancer Research, 2000, 6: 693−700.

62. Shin YK, Choi YL, Choi EY, *et al.* Targeted cytotoxic effect of anti−JL1 immunotoxin against a human leukemic cell line and its clinical implications. Cancer Immunol Immunother, 2003 Aug, 52: 506−512.

63. John M. Burke, MD, Joseph G, *et al.* Radioimmunotherapy for Acute Leukemia. Cancer Control, 2002, 9: 106−113.

64. Ghobrial I, Witzig T. Radioimmunotherapy: a new treatment modality for B−cell non−Hodgkin´s lymphoma. Oncology (Huntingt), 2004,18: 623−630.

65. Tobinai K. Monoclonal antibodies for the treatment of hematologic malignancies: clinical trials in Japan. Cancer Chemother Pharmacol, 2003 , 52: S90−96.

66. Alcindor T, Witzig TE. Radioimmunotherapy with yttrium−90 ibritumomab tiuxetan for patients with relapsed $CD20^{+}$ B−cell non−Hodgkin´s lymphoma. Curr Treat Options Oncol, 2002 Aug, 3: 275−282.

67. Bischof Delaloye A. The role of nuclear medicine in the treatment of non−Hodgkin´s lymphoma (NHL). Leuk Lymphoma, 2003, 44: S29−36.

68. Vose JM. Bexxar: novel radioimmunotherapy for the treatment of low−grade and transformed low−grade non−Hodgkin´s lymphoma. Oncologist, 2004, 9: 160−172.

69. Vandenbulcke K, De Vos F and Offner F, *et al.* In vitro evaluation of 213Bi−rituximab versus external gamma irradiation for the treatment of B−CLL patients: relative biological efficacy with respect to apoptosis induction and chromosomal damage. Eur J Nucl Med Mol Imaging, 2003, 30: 1357−1364.

70. Jurcic JG. Antibody therapy of acute myelogenous leukemia. Cancer Biother Radiopharm, 2000, 15: 319−326.

71. Scheinberg DA, Lovett D, Divgi CR, *et al.* A phase I trial of monoclonal antibody M195 in acute myelogenous leukemia: specific bone marrow targeting and internalization of radionuclide. J Clin Oncol, 1991, 9: 478−490.

72. Caron PC, Scheinberg DA. Anti−CD33 monoclonal antibody M195 for the therapy of myeloid leukemia. Leuk Lymphoma, 1993, 11: 1−6.

73. Schwartz MA, Lovett DR, Redner A, *et al.* Dose−escalation trial of M195 labeled with iodine 131 for cytoreduction and marrow ablation in relapsed or refractory myeloid leukemias. J Clin Oncol, 1993, 11: 294−303.

74. Caron PC, Schwartz MA, Co MS, *et al.* Murine and humanized constructs of monoclonal antibody M195 (anti−CD33) for the therapy of acute myelogenous leukemia. Cancer, 1994, 73: 1049−1056.

75. Jurcic JG, Caron PC, Nikula TK, *et al.* Radiolabeled anti−CD33 monoclonal antibody M195 for myeloid leukemias. Cancer Res, 1995, 55: 5908s−5910s.

76. McDevitt MR, Finn RD, Ma D, *et al.* Preparation of alpha−emitting ^{213}Bi−labeled antibody constructs for clinical use. J Nucl Med, 1999, 40: 1722−1727.

77. Sgouros G, Ballangrud AM, Jurcic JG, *et al.* Pharmacokinetics and dosimetry of an alpha−particle emitter labeled antibody: ^{213}Bi−HuM195 (anti−CD33) in patients with leukemia. J Nucl Med, 1999, 40: 1935−1946.

78. Burke JM, Jurcic JG, Scheinberg DA. Radioimmunotherapy for acute leukemia. Cancer Control, 2002, 9: 106−113.

79. Jurcic JG, Larson SM, Sgouros G, Targeted alpha particle immunotherapy for myeloid leukemia. Blood, 2002, 100: 1233−1239.

80. Burke JM, Caron PC, Papadopoulos EB, *et al.* Cytoreduction with iodine−131−anti−CD33 antibodies before bone marrow transplantation for advanced myeloid leukemias. Bone Marrow Transplant, 2003, 32: 549−556.

81. Jurcic JG, Divgi CCR, McDevitt MR, *et al.* Potential for myeloablation with yttrium −90 −HuM195 (anti −CD33) in myeloid leukemia. Proc Annu Meet Am Soc Clin Oncol, 2000, 19: 24

82. McDevitt MR, Ma D, Lai LT, *et al.* Tumor therapy with targeted atomic nanogenerators. Science, 2001, 294: 1537 −

1540.

83. Sievers EL. Targeted therapy of acute myeloid leukemia with monoclonal antibodies and immunoconjugates. Cancer Chemother Pharmacol, 2000, 46: S18−22.

84. Matthews DC, Appelbaum FR, Eary JF, *et al.* Phase I study of (131)I−anti−CD45 antibody plus cyclophosphamide and total body irradiation for advanced acute leukemia and myelodysplastic syndrome. Blood, 1999, 94: 1237−1247.

85. Vallera DA, Elson M, Brechbiel MW. Preclinical studies targeting normal and leukemic hematopoietic cells with Yttrium−90−labeled anti−CD45 antibody *in vitro* and *in vivo* in nude mice. Cancer Biother Radiopharm, 2003, 18: 133−145.

86. Waldmann TA, White JD, Carrasquillo JA. Radioimmunotherapy of interleukin−2R alpha−expressing adult T−cell leukemia with Yttrium−90−labeled anti−Tac. Blood, 1995, 86: 4063−4075.

87. Vallera DA, Elson M, Brechbiel MW, *et al.* Radiotherapy of CD19 Expressing Daudi Tumors in Nude Mice with Yttrium−90−Labeled Anti−CD19 Antibody. Cancer Biotherapy & Radiopharmaceuticals, 2004, 19: 11−23.

88. D Ma, MR McDevitt, E Barendswaard, *et al.* Radioimmunotherapy for model B cell malignancies using ^{90}Y−labeled anti−CD19 and anti−CD20 monoclonal antibodies. Leukemia, 2002, 16: 60−66.

89. Seitz U, Neumaier B, Glatting G, *et al.* Preparation and evaluation of the rhenium−188−labelled anti−NCA antigen monoclonal antibody BW 250/183 for radioimmunotherapy of leukaemia. Eur J Nucl Med,1999, 26: 1265−1273.

90. Kotzerke J, Glatting G, Seitz U, *et al.* Radioimmunotherapy for the intensification of conditioning before stem cell transplantation: differences in dosimetry and biokinetics of ^{188}Re−and ^{99m}Tclabeled anti−NCA−95 MAbs. J Nucl Med, 2000, 41: 531−537

91. Bunjes D,Buchmann I,Duncker C, *et al.* Rhenium 188−labeled anti−CD66 (a, b, c, e) monoclonal antibody to intensify the conditioning regimen prior to stem cell transplantation for patients with highrisk acute myeloid leukemia or myelodysplastic syndrome: results of a phase I−II study. Blood, 2001, 98: 565−572

92. Dearden C. Monoclonal antibody therapy of haematological malignancies. BioDrugs, 2002, 16: 283−301.