

Overview of monoclonal antibodies in cancer therapy: present and promise

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Abstract

After 30 years of development, therapy with monoclonal antibodies has started to realize its promise. Clinical use is most widespread in the field of oncology, where half of the agents approved for routine clinical use are employed and a large number of molecules are currently undergoing clinical trials. In the past 2 years alone, three new compounds—the radiolabeled antibody ^{131}I -tositumomab and two antibodies targeting growth factor receptors (bevacizumab and cetuximab)—have received FDA approval for indications in oncology.

This review summarizes the development of this exciting treatment modality over the last three decades, examines the outcome of treatment with these new antibodies and others available for routine clinical use (i.e. rituximab, trastuzumab, alemtuzumab, gemtuzumab ozogamicin, ^{90}Y -ibritumomab tiuxetan) in standard indications and in experimental settings, and gives a brief outlook on possible future developments.

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1. Introduction

The idea of the existence of a soluble serum content with activity against pathogens and toxins dates back to the final years of the 19th century when Behring detected that the lethal effects of the administration of large amounts of tetanus toxin to rabbits could be prevented by immunising animals with serum obtained from other rabbits which had before been treated with a minimal non-lethal dose of toxin. Behring concluded correctly that the serum must contain an anti-toxin with specific activity against tetanus toxin [1].

While serotherapy to treat tetanus and diphtheria was rapidly developed in the following years, it was one of Behring's colleagues, Ehrlich, who at the turn of the century first reasoned on the possibility of extending the then already widespread use of serotherapy to treat malignant disease [2].

Hericourt and Richet pioneered this approach by preparing anti-serum to extracts of osteogenic sarcoma in animals and treating more than 50 patients with osteogenic sarcoma with encouraging results [3].

After such a promising start, it took many decades, however, until in 1968 the structure of the assumptive anti-toxin was characterized by Porter [4] and Edelman et al. [5] to be the immunoglobulin with its today universally known Y-shaped structure. Only little later, in 1975, Kohler and Milstein published results of their work describing the production of “hybridoma” cell lines capable of producing monoclonal antibodies (MAbs): polyclonal antibodies are produced in animals repeatedly immunized with the target antigen; the antibody-producing B-lymphocytes are then isolated and immortalized by hybridisation with a murine myeloma cell line [6].

The rapidly developing technique soon entered clinical trials: in 1980, the first patient with relapsed lymphoma was treated. The antibody which had shown to lyse the patient's tumour cells in vitro turned out to be clinically ineffective due to large amounts of circulating target antigen, but therapy was safe and well-tolerated [7]. Comparable trials in cancer patients treated with these antibodies found only short-lived responses [8]. One notable exception was a patient with B-cell lymphoma achieving a complete remission after treatment with murine antibodies recognizing the unique immunoglobulin expressed on the patient's malignant B-lymphocytes (anti-idiotypic antibodies) [9]. Even though the immunoglobulin receptor proved to be the perfect target for monoclonal antibodies due to its selective expression on malignant cells—some of the patients in treated in the early 1980s are still in continuous complete remission—this technique, involving the creation of antibodies specific for each patient, proved to be too cumbersome and expensive for routine clinical use.

Further research revealed several reasons why generic murine antibodies failed to produce durable remissions:

- lack of highly specific tumour antigens;
- short circulating half-life;
- limited penetration into tumour sites;
- inadequate recruitment of host effector functions;
- internalisation of target antigens by tumour cells;
- production of neutralizing human anti-mouse antibodies;
- inadequate quantities of antibody administered (in the milligram range) due to limitations of the production process.

The search for the optimal target and the struggle to overcome these obstacles lasted the following 15 years. After

Table 1
Monoclonal antibodies in the order of FDA-approval [124]

FDA approved	Product	Target	Type	Indication
1986	Muromonab (Orthoclone OKT3®)	CD3	Murine IgG2a	Transplant rejection
1994	Abciximab (ReoPro®)	GP IIb/IIIa	Chimeric Fab	Prevention of re-stenosis after PTCA
1997	Daclizumab (Zenapax®)	CD25	Humanized IgG1	Transplant rejection
1997	Rituximab (Rituxan®)	CD20	Chimeric IgG1	B-cell lymphoma
1998	Basiliximab (Simulect®)	CD25	Chimeric IgG1	Transplant rejection
1998	Palivizumab (Synagis®)	RSV	Humanized IgG1	RSV bronchiolitis
1998	Infliximab (Remicade®)	TNF	Chimeric IgG1	Crohn's disease, rheumatoid arthritis
1998	Trastuzumab (Herceptin®)	HER2/neu	Humanized IgG1	Breast cancer
2000	Gemtuzumab (Mylotarg®)	CD33	Humanized IgG4-toxin-conjugate	Acute myeloid leukaemia
2001	Alemtuzumab (MabCampath®)	CD52	Humanized IgG1	Chronic lymphatic leukaemia
2002	⁹⁰ Y-ibritumomab (Zevalin®)	CD20	Murine IgG1-radionuclide-conjugate	B-cell lymphoma
2002	Adalimumab (Humira®)	TNF	Human IgG1	Rheumatoid arthritis
2003	Omalizumab (Xolair®)	IgE	Humanized IgG1	Asthma
2003	¹³¹ I-tositumomab (Bexxar®)	CD20	Murine IgG1-radionuclide-conjugate	B-cell lymphoma
2003	Efalizumab (Raptiva®)	CD11a	Humanized IgG1	Psoriasis
2004	Bevacizumab (Avastin®)	VEGFR	Humanized IgG1	Colorectal cancer
2004	Cetuximab (Erbix®)	EGFR	Chimeric IgG1	Colorectal cancer

early enthusiasm in the 1970s and 1980s, therapeutic monoclonal antibodies were able to establish themselves as one of the most important and fastest growing classes of therapeutic drugs in the second half of the last decade with 17 antibodies approved by the FDA today, 8 of them used in malignant disease (see Table 1).

In order to recapitulate the development, we will shortly review the structure of physiologically occurring immunoglobulins.

2. Structure and function of human antibodies

Immunoglobulins or antibodies are Y-shaped molecules with a molecular weight of around 150 kDa consisting of four covalently bound elements—two light chains and two heavy chains. Both heavy and light chains have a constant part, which is identically found in all immunoglobulins of the same isotype, and a unique variable part containing the antigen-binding site located on the two short arms of the molecule.

By cleavage with papain, a so-called “Fab” or “fragment antigen binding” part can be separated from the “Fc” or “fragment crystalline” part of the molecule.

The Fab fragments contain the variable domains, which themselves consist of three hypervariable amino acid sequences responsible for the antibody specificity (the “complementarity determining regions” or CDR) embedded into largely constant “framework regions”.

The Fc portion of the molecule contains only constant parts of the two heavy chain molecules. It determines the isotype of the antibody (IgA, IgD, IgE, IgG, or IgM) and serves as a binding site for complement and leukocytes.

Circulating antibodies protect the host organism in two ways:

- by directly binding and neutralizing toxins;

- by activating the host immune response, mainly through two pathways:
 - o binding and activation of complement C1q on the Fc region resulting in the formation of the membrane attack complex lysing the target cell (“complement mediated cytotoxicity”);
 - o initiation of a cellular response (“antibody dependent cellular cytotoxicity”) occurring through binding of Fc receptors (CD16, CD32, and CD64) on natural killer cells, monocytes and macrophages to the opsonized target.

Different isotypes and subclasses differ in their ability to activate this response: while human IgM antibodies almost exclusively activate complement, IgG isotypes (and especially the IgG1 and IgG3 subclasses) lead to strong antibody-dependent cellular cytotoxicity.

3. Structure and function of therapeutic antibodies

The murine antibodies originally used suffered from two major shortcomings. Their rodent origin led to recognition by the host immune system and to the development of human anti-mouse antibodies (HAMA). These HAMA not only inactivated and eliminated murine antibodies after repeated administration, the formation of antibody-HAMA-complexes also led to allergic reactions up to anaphylactic shock, largely limiting the possibility of repeated administration.

Due to differences between the murine and the human immune system, the mouse Fc portion also impaired the possibility of inducing complement mediated and antibody-dependent cellular cytotoxicity, restraining therapeutic possibilities.

To overcome these issues, the originally murine molecules were progressively engineered with the goal of minimiz-

ing immunogenic content and allowing the triggering of immunologic efficiency.

In a first step, the constant domains of the murine molecule were replaced by their respective human counterparts, leading to a so-called chimeric antibody. Human gene sequences were typically taken from the kappa light chain and the IgG1 heavy chain. The IgG1 subclass was selected, as it is most efficient in activating complement and cytotoxic effector cells.

As an additional benefit, by replacing roughly two-thirds of the molecule with human protein, the resulting molecule was reduced in its immunogenicity allowing a prolonged serum half-life and multiple applications.

As a refinement of this strategy, humanized antibodies were engineered, by grafting murine CDR into human antibodies, resulting in a molecule of roughly 95% human origin. All of the eight approved antibodies used in oncological indications and the majority of molecules in advanced clinical trials are of chimeric or humanized structure.

Further development has led to the production of fully human antibodies. As humans cannot be vaccinated to produce antibodies to self-antigens, different methods were developed including the transfer of immunoglobulin genes into mice, which can subsequently be vaccinated leading to production of human antibodies (the HuMab MouseTM) or completely bypassing the need for immunization by production of antibodies in phage display libraries. An excellent review on different methods to produce fully human antibodies was recently published [10]. No fully human antibody has yet been approved for the treatment of cancer, but for new antigens with no existing mouse MAbs, the direct production of human antibodies today offers the fastest pre-clinical development.

4. Rituximab

Non-Hodgkin lymphoma (NHL) was the first malignant disease where single patients were successfully treated with monoclonal antibodies. It also represents the malignancy where treatment success could first be reproduced on a large scale.

The development of rituximab, a chimeric IgG1 monoclonal antibody against the CD20 antigen, serves as an example of the importance of choosing an optimal target in antibody-based therapy of cancer. CD20 is trans-membrane phosphoprotein with the following properties:

- CD20 is densely expressed on the majority of B-cell lymphoma cells, but not on most other cells of the body.
- CD20 is expressed on most B-lymphocytes excluding immature precursor cells (allowing reconstitution of the B-cell compartment after therapy) and plasma cells (allowing the continuous production of immunoglobulins).
- CD20 is not shed into the plasma after binding of rituximab, nor is it internalised after antibody engagement.
- CD20 is essential in the differentiation and proliferation of B-lymphocytes [11].

- Apart from the well known general mechanism of action (i.e. antibody-dependent cellular cytotoxicity and complement-mediated cytotoxicity), the binding of rituximab to CD20 leads to a calcium influx and apoptosis [12].

Administration of rituximab causes rapid depletion of normal and malignant CD20-positive B-lymphocytes from blood, bone marrow and lymph nodes of the recipient lasting between 3 and 6 months following the last administration. Importantly, rituximab does not compromise humoral immunity: despite B-lymphopenia and a significant drop of plasma IgM levels, the incidence of infections is not increased even after prolonged treatment with the antibody [13].

Rituximab has a distinctive infusion related toxicity profile. Side effects include fever, chills, hypotension and nausea in a majority of patients during and in the first hours after the initial administration, and at a greatly reduced frequency and intensity during the following applications. The intensity of the adverse reactions correlates with the number of circulating malignant cells, suggesting the lysis of normal and malignant B-lymphocytes and the ensuing release of cytokines is responsible for infusion associated toxicity [14]. Symptoms can be alleviated by the pre-infusion administration of acetaminophen and an antihistaminic agent and typically respond to temporary discontinuation of the infusion.

More severe infusional toxicity includes bronchospasm, angioedema and acute lung injury often associated with high circulating cell counts or pre-existing cardiac or pulmonary disease. Patients at risk should receive additional pre-medication with steroids, which prevents the detrimental effects of overwhelming cytokine release.

4.1. Rituximab in follicular lymphoma

Relapsed indolent non-Hodgkin lymphoma and most prominently follicular lymphoma (FL) is the most extensively studied indication for the use of a monoclonal antibody in oncology. The clinical development led from successful single dose application in patients refractory to chemotherapy [15], to repeated weekly administration of escalating doses of up to 375 mg/m² [16,17]: half of the 37 patients treated achieved remission, a minority (9%) of them complete remission (CR), while 41% were in partial remission (PR) at the end of treatment. Median progression free survival (PFS) was 11.8 months in patients responding to therapy. A pivotal multi-centre phase II study including 166 patients confirmed the results with an overall remission rate of 50% (6% CR, 44% PR) and a median time to progression of 12.5 months [18]. The results of this trial led to FDA approval of the drug for relapsed/refractory low-grade NHL.

4.1.1. Re-treatment

Patients relapsing after successful treatment with rituximab were retreated in a trial with 58 patients [19]. Response rates were comparable (11% complete response, 30% partial response) to first treatment as were adverse side effects. The

median time to progression was longer than after the first treatment (17.8 months). Coiffier et al. followed a group of 59 patients who were re-treated with rituximab either alone or in combination with chemotherapy [20]: overall response rate of first rituximab treatment had been 89%. Ninety-three percent showed a response to re-treatment, median time to progression was also prolonged compared to the initial treatment (20 months versus 12 months), possibly as a result of selection bias, as only a minority of patients relapsing after the first treatment received rituximab re-treatment. Interestingly, 12 of the 20 patients that progressed after rituximab re-treatment received a third treatment and all of them responded again.

4.1.2. Maintenance and prolongation of treatment

Due to its acceptable toxicity profile and the success in re-treatment, the question whether rituximab maintenance treatment is able to improve response rates and prolong remission duration is of considerable interest.

The main argument in favour of this approach is the strong correlation between rituximab plasma levels and response rate seen in the pivotal trial: while responders typically had measurable serum levels of rituximab for 3–6 months, patients failing to respond cleared rituximab much faster from their circulation [21]. As mean serum antibody concentration was inversely correlated with the presence of bulky disease and the number of circulating B-cells, it was proposed that patients with a higher tumour load might need higher doses or prolonged treatment to be able to achieve the necessary serum levels.

A randomised trial suggests that adding maintenance doses of rituximab prolongs response duration [13]: 202 patients with newly diagnosed or relapsed follicular lymphoma were treated with standard rituximab ($375 \text{ mg/m}^2 \times 4$). Patients achieving stable disease, partial or complete remissions at the end of induction therapy were randomised to receive either four additional maintenance doses every 8 weeks or no further treatment. Median event-free survival was prolonged in the groups receiving maintenance treatment (23 months versus 12 months in the control group) suggesting a benefit derived from the continued treatment.

In another trial, prolongation of standard treatment from 4 to 8 weekly doses did not lead to a higher response rate but prolonged response duration: in 37 patients with relapsed or refractory low-grade lymphoma median time to progression and response duration were prolonged to >19.4 and >13.4 months, respectively [22].

To find out whether rituximab maintenance offers any benefit compared to re-treatment in case of relapse, 114 patients with indolent NHL responding to standard 4-week treatment were randomised to receive maintenance treatment (4 weekly doses every 6 months for a maximum of 2 years) versus re-treatment at the time of progression [23]. After a median follow-up of 41 months, more patients in the maintenance group remained in continuous complete remission (45% versus 24%), but median duration of rituximab benefit was simi-

lar in both groups (31 months versus 27 months, respectively). Further follow-up will determine if the increased number of continuous remissions will result in overall benefit for the patients receiving maintenance treatment.

4.1.3. Combination with chemotherapy

Based on in vitro data suggesting synergistic action of rituximab and cytotoxic agents including anthracyclines [24], the combination of six cycles of chemotherapy consisting of CHOP (cyclophosphamide, adriamycin, oncovin and prednisone) with concurrently administered rituximab in 40 patients with predominantly untreated follicular lymphoma greatly increased the response rate (55% CR, 40% PR) suggesting synergistic action with no significant toxicity added [25]. Median time to progression was recently determined to be 82.3 months [26]. Results are comparable if, instead of parallel administration, CHOP is followed by rituximab [27].

The combination of rituximab with chemotherapy containing purine analogues such as fludarabine further increases CR rate to 80% [28]. The superiority in achieving CR of combinations containing purine analogues is supported by a trial published in abstract form comparing fludarabine/mitoxantrone with rituximab consolidation to CHOP/rituximab showing CR rates of 87 and 76%, respectively [29].

Adding rituximab to chemotherapy is strongly associated with achieving molecular remission: 15 of 17 patients with advanced follicular lymphoma attained a molecular response after treatment with mitoxantrone, chlorambucil and prednisolone (MCP) combined with rituximab, compared to 0/12 molecular remissions achieved after chemotherapy alone [30]. It must be stressed, however, that neither the achievement of CR nor molecular response guarantees prolonged complete remission: after treatment with fludarabine, cyclophosphamide and rituximab 8/28 patients in CR and 5/19 patients achieving molecular response were subject to a relapse after a median follow-up of 13 months [31].

The combined toxicity of immuno-chemotherapy regimens in heavily pre-treated patients, especially those combining rituximab with a purine analogue leading to a prolonged combined T- and B-lymphocyte depletion, has to be considered when evaluating responses achieved with these combinations: it is worth recording that one study in 17 patients receiving rituximab + fludarabine and cyclophosphamide had to be prematurely closed due to increased haematological toxicity and infectious complications [32].

4.1.4. Combination with cytokines

An alternative to the addition of chemotherapy to rituximab is the combination with interferon- α -2a, which has both direct anti-lymphoma activity, increases CD20 antigen expression on lymphoma cells, and potentially augments the immune response evoked by rituximab. A phase II study included 42 patients with relapsed or refractory indolent B-cell NHL treated with a combination of four doses of rituximab and interferon alpha at a dose of 2.5–5 Mio units three

times weekly. The overall response rate was 45%, comparable to that seen with single agent rituximab therapy [33]. Time to progression however, was roughly doubled to 23 months compared to historical controls. The increase in response duration was obtained at the price of increased toxicity, notably asthenia, chills, fever, headache, nausea, and myalgia in the majority of patients. Combination with interferon can therefore at the moment not be recommended as standard therapy.

Trials combining rituximab with other cytokines such as interleukin-2 [34], interleukin-12 [35] and GM-CSF [36] have shown promising preliminary results in Phase I studies with overall response rates of up to 80% and CR rates in the range 40%. Phase II studies are currently underway to confirm these data in larger patient populations.

4.1.5. First-line therapy

Recently, there has been increasing interest in using rituximab as a first-line therapy. Arguments in favour of this approach include its excellent toxicity profile, the presumed low rate of secondary malignancy and its lack of stem cell toxicity. In a phase II study, 62 patients with indolent NHL—the majority of them follicular lymphoma—were treated with first-line rituximab in the standard dose of 375 mg/m² for 4 weeks [37]. Patients achieving an objective remission received maintenance treatment of rituximab every 6 months for a maximum of 2 years. The response rate of 73% (CR 37%, PR 36%), as well as the time to progression (34 months), was clearly superior to responses seen in pre-treated patients. Similar results were seen in 49 patients with follicular lymphoma and low tumour burden treated with the standard dose for 4 weeks: overall response rate was 73% (CR 27%) [38].

The increase in efficacy compared to that seen in pre-treated patients is consistent with the inverse relationship between the response to rituximab mono-therapy and the number of prior relapses that was observed in earlier trials [18]. Future trials should be carried out to establish its role in first-line therapy; however, accrual in randomised trials has become difficult. Despite the lack of data from randomised trials, it is estimated that currently more than 80% of patients newly diagnosed with follicular lymphoma in the US and treated outside clinical trials receive rituximab as part of their first-line treatment [39], and the number patients willing to participate in a study possibly confining them to chemotherapy alone is decreasing.

4.2. Rituximab in low-grade lymphoma other than FL

The majority of patients with low grade NHL treated with rituximab mono-therapy in clinical trials suffered from follicular lymphoma. Treatment of other entities has proved to be less successful with response rates ranging from 14% in B-chronic lymphatic leukaemia (CLL) to 28% in lymphoplasmocytic lymphoma and 37% in mantle cell lymphoma (MCL) [40].

The most extensive data exist for mantle cell lymphoma, a malignancy with low-grade histological characteristics that tends to pursue an aggressive clinical course and is incurable by standard chemotherapy. Response rates to single agent rituximab are as noted above with a median response duration of 1 year.

An interim analysis of a randomised trial comparing FCM (fludarabine/cyclophosphamide/mitoxantrone) to FCM/rituximab in 38 patients with MCL has shown a striking increase in remission rate (overall response 65% versus 33%; CR 35% versus 0%) [41]. A trend towards longer overall and disease-free survival for rituximab plus FCM has been observed, but longer follow-up is required. About one in three patients achieved a molecular remission suggesting rituximab might be added to standard chemotherapy for in vivo purging before autologous stem cell transplantation. The role of rituximab in this setting is reviewed below.

CLL is a low-grade NHL that typically shows dim expression of CD20 in flow-cytometry. Response rates of CLL and its non-leukaemic counterpart small lymphocytic lymphoma (SLL) to standard dose single-agent rituximab are in the range of 10–15% [18]. Responses are typically of short duration.

Initially, the lower expression of CD20 on CLL/SLL cells compared to other indolent lymphoma cells was suspected to be the reason for poor response rates. Recent data suggest however that the level of expression of CD20 does not affect the response rate [42]. Increased levels of soluble CD20 circulate in the serum of CLL patients [43], presumably neutralizing rituximab before it can reach its target cells and leading to lower serum levels, which are known to correlate with short response duration.

To increase the poor response rate of rituximab, a trial administering escalating doses of rituximab up to 2250 mg/m² four times in weekly intervals was conducted [44]. Toxicity was comparable to that seen after standard doses and the response rate increased with rising doses up to 75% in patients in the highest dose group. Median duration of response was 9.5 months. Similar results can be achieved by thrice weekly administration of standard dose (375 mg/m²) rituximab [45].

An alternative to enhance the response rate of rituximab in CLL—and one that is less prohibitive from an economical and practical point of view—is combination with chemotherapeutic agents. FCR (fludarabine, cyclophosphamide, rituximab) has shown a response rate of 100% in 202 patients with untreated CLL (68% CR, 18% nodular partial remission [i.e. CR except for residual lymphoid aggregates on bone marrow biopsy], and 14% PR) [46] and 56% in pre-treated patients (14% CR) [47]. The combination of fludarabine and rituximab shows similar efficacy [48].

Other less frequent diseases where rituximab has been used successfully include entities such as lymphocyte predominant Hodgkin disease [49], post-transplant lymphoproliferative disease [50] and a range of autoimmune dis-

orders. The use of rituximab in B-cell disorders other than non-Hodgkin lymphoma has recently been reviewed [51].

4.3. Rituximab in high-grade lymphoma

Contrary to indolent lymphomas, patients with highly aggressive lymphoproliferative disease have a considerable chance of being cured after chemotherapy. As the majority of high-grade mature B-cell neoplasias express CD20, the addition of rituximab to standard poly-chemotherapy is an evident approach to increase cure rate without escalation of toxicity.

A large French trial (GELA) in 399 patients ≥ 60 years with previously untreated diffuse large-B-cell lymphoma comparing eight cycles of standard chemotherapy with CHOP given at intervals of 3 weeks to a combination of CHOP with rituximab administered on day 1 of each cycle resulted in significantly superior response rates (76% versus 63%, $P = 0.005$) and survival after 2 years of follow-up (70% versus 57%, $P = 0.007$) [52]. No increase in toxicity compared to standard chemotherapy was observed.

Interestingly, the addition of rituximab to CHOP seems to overcome the traditionally poor prognosis of patients with strong expression of bcl-2 [53] (see Table 2).

These data also show that the survival benefit of the addition of rituximab primarily stems from the roughly two thirds of patients with bcl-2-positive lymphoma. If this is confirmed in future trials, addition of rituximab to standard treatment might someday be limited to patients with bcl-2-positive neoplasia.

Alternative attempts to enhance cure rate in aggressive lymphoma include increasing dose density by administering standard doses of CHOP at intervals of 14 days instead of the standard 21 days. Recent trials have shown that this is feasible with G-CSF support and increases survival in patients with aggressive B-cell lymphoma in the same range as the improvement achieved by the addition of rituximab [54]. Whether the addition of rituximab to CHOP-14 confers any additional benefit is currently being investigated by the German High-Grade non-Hodgkin Lymphoma Study Group.

The superiority of the R-CHOP combination to chemotherapy alone was confirmed in patients < 60 years in the MINT-trial, which was stopped prematurely after an interim analysis after a median follow-up of 15 months showed a significantly higher complete remission rate (84.7% versus 66.0%) and a lower rate of progressive disease (6.3% versus 17.7%) [55]. An interesting retrospective study analysed the outcome of 293 patients with diffuse large B-cell lymphoma

treated in British Columbia in the 18 months before and after the standard practice was changed from CHOP to R-CHOP. Two-year overall survival increased from 40 to 67% in patients ≥ 60 years and from 69 to 87% in patients < 60 years [56].

4.4. Rituximab in high-dose chemotherapy

High dose chemotherapy with autologous stem cell rescue is a valuable salvage therapy in patients with relapsing NHL. Rituximab has been used as an in vivo purging agent both before and after stem cell transplantation. In a remarkable German multi-centre Phase II study [57], 20 patients with stage III/IV follicular lymphoma with $t(14;18)$ and 10 patients with mantle cell lymphoma with $t(11;14)$ were treated with CHOP or a similar chemotherapy regimen. Patients achieving a remission were auto-transplanted after conditioning with total body irradiation and cyclophosphamide. Four doses of standard dose rituximab were administered between weeks 8 and 11 post-transplant.

CR rates developed over time, being 57% at 6 months and 88% at 12 months. After 2 years, 29 of 30 patients were in CR, 1 patient with MCL had relapsed. The number of molecular responses had gradually increased from 22% pre-transplant to 53% after conditioning to 72% 4 weeks after rituximab therapy to 100% at 6 months after transplantation.

Similar data come from an Italian trial including 28 patients with previously untreated mantle cell lymphoma: 54 months after high-dose chemotherapy with co-administration of rituximab as an in vivo purging agent, survival was 89% [58], suggesting this approach might alter the natural course of the disease.

In general rituximab appears to be well tolerated in the setting of autologous stem cell transplantation and does not affect cellular recovery or the post-transplant levels of immunoglobulins [59,60].

5. Alemtuzumab

Alemtuzumab or Campath-1H is a humanized rat-IgG1-antibody binding to CD52, an antigen with unknown function found on $> 95\%$ of peripheral blood lymphocytes and monocytes and to a smaller extent on granulocytes and in the male genital tract. Most B- and some T-cell lymphomas highly express CD52, whereas the protein is absent on bone marrow progenitor cells.

Campath-1H is the final product of a development initiated more than two decades ago and exemplifying the maturation of monoclonal antibodies from their first experimental use to widespread clinical application today.

Initially developed as a rat IgM-antibody (Campath-1M) successfully applied for in vitro T-cell depletion to prevent graft-versus-host disease [61], the antibody was soon administered to individual patients with lymphoma. Results were disappointing: the antibody produced transient deple-

Table 2
Response to CHOP and R-CHOP with respect to bcl-2-expression

2-Year survival	CHOP (%)	R-CHOP (%)	
Bcl-2-positive	48	67	$P = 0.004$
Bcl-2-negative	67	72	$P = 0.13$

tion of blood lymphocytes with consumption of complement, but had no effect on solid tumour masses or bone marrow [62]. The failure of Campath-1M was attributed to its IgM-isotype allowing complement activation but not antibody dependent cytotoxicity. The molecule was therefore further engineered resulting in Campath-1G, a rat IgG2b antibody with the ability to activate human Fc-receptors and thereby induce ADCC. Contrary to Campath-1M, the IgG2b antibody produced long-lasting depletion of lymphocytes from blood and marrow and improvement of splenomegaly. Due to its murine structure however, patients quickly developed human-anti-mouse-antibodies (HAMA), which effectively neutralized Campath-1G after repeated administration [62].

In 1988, Campath was humanized and the resulting molecule was called Campath-1H or alemtuzumab. The first results in individual patients with advanced B-cell lymphoma were promising enough to warrant further studies [63]. In the following years, however, development of alemtuzumab was mainly oriented to patients with autoimmune diseases such as multiple sclerosis or rheumatoid arthritis. Treatment with Campath proved to be effective, but the rate of side effects seemed intolerable and development of the compound came to a halt in 1994. It was not until the end of the decade that it re-appeared after encouraging results were seen in patients with CLL.

A phase II trial in 50 patients with low-grade lymphoma refractory to chemotherapy or in relapse were treated with Campath-1H 30 mg i.v. three times weekly for a maximum of 12 weeks [64]. Results were promising with a pattern that would be seen throughout the future trials: malignant cells were rapidly eliminated from blood in >90% of patients. CR in the bone marrow was obtained in 32% of the patients, whereas lymphadenopathy normalized in only 5%.

Toxicity was substantial: all patients suffered from profound lymphopenia and the rate of grade IV neutropenia was 28%. Accordingly, the rate of infection and death from infection was significant: localised reactivation of herpes simplex in 44% of patients and oral candidiasis (26%) were among the most common. Pneumonia was diagnosed in 28% of patients, one patient died from pneumocystis carinii infection. Other pathogens causing pneumonia included cytomegalovirus (6%), aspergillus (4%), and *M. tuberculosis* (2%).

Infusion related toxicities included fever, rigors and chills in more than 80% of patients leading to a dose escalation regimen in future trials: daily doses of 3 mg are administered until infusion related side effects are of grade 2 or lower. The daily dose is then escalated to 10 mg and continued until infusion reactions are again tolerated. Eventually, the maintenance dose of 30 mg is administered three times weekly.

Among the most promising subgroups of non-Hodgkin lymphoma responding to Campath-1H were patients with chronic lymphocytic leukaemia and T-prolymphocytic-leukaemia, making these entities the natural subject of the following trials.

5.1. Alemtuzumab in CLL

Ninety-three patients with CLL after prior therapy with an alkylating agent and without response to fludarabine were treated with increasing doses of Campath-1H (target dose 30 mg, three times weekly for a maximum of 12 weeks). Infection prophylaxis was mandatory, beginning on day 8, and continuing for a minimum of 2 months after treatment [65]. Overall response was 33% (2% CR, 31% PR) and median time to progression was 9.5 months for responders.

Despite infection prophylaxis, grade 3 or 4 infections were reported in 27% of the heavily pre-treated patients including pneumocystis and aspergillus pneumonia, systemic candidiasis, cryptococcal pneumonia and listeria meningitis leading to death in 11 patients.

Based on these data and other trials showing similar results [66,67], the drug received orphan drug status and FDA-approval for the treatment of CLL refractory to fludarabine was attained in 2000.

Preliminary data on six CLL patients refractory to both single agent alemtuzumab and fludarabine found that five of them responded to a combination treatment resulting in reasonable response durations, offering a therapeutic possibility for patients refractory after alemtuzumab mono-therapy [68].

Combination of alemtuzumab with other monoclonal antibodies like rituximab is another approach to enhance response rates. In the largest retrospective analysis including 48 patients with relapsed or refractory NHL—the majority of them CLL—overall response rate achieved by combination of rituximab/alemtuzumab was 52% [69]. The rationale of combining the two antibodies is supported by evidence that the acquirement of resistance to rituximab is associated with up-regulation of the CD52 antigen [70].

While all the above data concern patients treated intravenously with alemtuzumab, a small case series published 1997 demonstrated that the subcutaneous administration of alemtuzumab in seven patients had comparable efficacy without the infusion related toxicity seen after i.v. administration [71]. The markedly superior toxicity profile was later reproduced in 41 patients with CLL receiving alemtuzumab as first-line treatment [72]. Overall response rate was 87% (19% CR, 68% PR); the median response duration has not been reached after 18 months. Rigor, rash, nausea, dyspnoea, and hypotension were rare or absent. Transient grade IV neutropenia developed in 21% of the patients. Infections were less frequent than seen in patients refractory to chemotherapy, but 10% patients developed CMV reactivation. How these encouraging results compare to the data achieved in pre-treated patients receiving intravenous alemtuzumab will have to be investigated in future trials.

As with other monoclonal antibodies, treatment with alemtuzumab might be most effective in patients with minimal tumour load. In a phase III trial in CLL patients in first remission after treatment with fludarabine or fludarabine/cyclophosphamide, 11 patients were randomised to receive consolidation with alemtuzumab while 10 patients re-

ceived no further treatment. Progression free survival in the control group was 24.7 months, while no progression has yet been noted in the group receiving alemtuzumab [73]. However, patient accrual was stopped prematurely due to grade 3/4 infections occurring in 7/11 patients receiving alemtuzumab.

5.2. Alemtuzumab in T-PLL

Apart from CLL the most promising indication for alemtuzumab is T-prolymphocytic leukaemia. T-PLL is a rare aggressive malignancy of mature T cells with a median survival of 7.5 months. No standard therapy is established. Already in 1997, extraordinary response rates were noted after treatment with alemtuzumab (CR in 60% versus 12% CR in patients treated with conventional chemotherapy) [74] and the drug has since been available through a compassionate use programme. A retrospective analysis of 76 patients with T-PLL treated with alemtuzumab showed an overall response rate of 51% with 40% complete responses [75]. The median duration of CR was short however, with 8.7 months, unsatisfactory if no other treatment follows, but sufficient to open a window of opportunity for allogeneic stem cell transplantation in patients eligible for this type of treatment.

6. Trastuzumab

The proto-oncogene human epidermal growth factor receptor 2 (HER2, also referred to as HER2/neu or c-erbB-2) encodes the HER2-receptor, a transmembrane protein found on numerous epithelial tissues and over-expressed on 20–30% of breast cancers [76]. Over-expression of the membrane receptor is most commonly caused by gene amplification [77], which leads to an increase in the production of HER2-mRNA and eventually of HER2-protein. While a normal breast epithelial cell may carry around 20–50,000 HER2-receptors on its surface, the presence of up to 2 million receptors per cell has been reported in patients with HER2-overexpressing breast cancer [78].

Over-expression of HER2 correlates with hormone receptor negativity, a reduced sensitivity to hormone therapy and chemotherapy (with the possible exception of anthracycline based treatment [79]) and worse survival, suggesting an essential position of HER2 in oncogenesis. With this in mind, the humanized monoclonal antibody HER2-antibody trastuzumab was developed.

6.1. Trastuzumab single agent therapy in metastatic breast cancer

An early phase II study involving 46 patients with heavily pre-treated HER2-over-expressing metastatic breast cancer treated with trastuzumab single agent therapy weekly for 10 weeks resulted in one complete remission and four partial remissions (OR rate, 11.6%) [80]. An additional 14 patients (37%) showed minimal response or stable disease with

a median time to progression of 5.1 months. Results were confirmed in an international open-label study including 222 patients with HER2-over-expressing metastatic breast cancer that had progressed after one or two chemotherapy regimens [81]. HER2-status was assessed by immunohistochemistry. Patients were treated with trastuzumab single agent therapy with a loading dose of 4 mg/kg followed by weekly doses of 2 mg/kg. Overall response rate was 15% (CR 4%, PR 11%) with a median duration of response of 9.1 months. The results of this trial led to FDA approval of trastuzumab single agent therapy as second-line treatment for HER2-positive metastatic breast cancer.

6.2. First-line single agent therapy

One trial investigated the use of trastuzumab as first-line single agent therapy in metastatic breast cancer. Patients were randomised to two groups receiving either standard dose (4 mg/kg initial dose followed by 2 mg/kg weekly) or twice the standard dose (8 mg/kg initial dose followed by 4 mg/kg weekly) and treated until progression [82]. Of 112 evaluable patients, CRs were noted in 6 patients (5.3%) and 20 achieved a PR (18%) for an overall response rate of 23%, comparable to that seen in pre-treated patients. No difference in efficacy was seen between standard dose and increased dose therapy.

6.3. Trastuzumab combined with chemotherapy in metastatic breast cancer

In the largest comparative trial, 469 patients were randomly assigned to receive either standard chemotherapy or standard chemotherapy plus trastuzumab [83]. Patients without prior anthracycline therapy were treated with a combination of doxorubicin or epirubicin and cyclophosphamide (AC or EC, 138 women) alone or with trastuzumab (143 women). Patients with a history of anthracycline exposure were treated with paclitaxel alone (96 women) or paclitaxel with trastuzumab (92 women). Patients received trastuzumab weekly until disease progression; chemotherapy was administered every 3 weeks for at least six cycles. Response rates and duration are shown in the Table 3.

In summary, there were 118 responses in the chemotherapy/trastuzumab groups (50%) compared to only 74 responses in the chemotherapy groups (32%). Addition of trastuzumab to chemotherapy prolonged the median survival by 4.8 months, from 20.3 to 25.1 months, a result that is even more remarkable in light of the fact that 72% of patients originally randomised to chemotherapy treatment received trastuzumab after progression of their disease.

Laboratory data from cell cultures suggest synergism between trastuzumab and a range of chemotherapeutic agents [84] including vinorelbine, docetaxel, cisplatin and etoposide. Addition of effects was seen in combinations of trastuzumab with epirubicin, doxorubicin, paclitaxel, methotrexate and vinblastine, while the combination 5-FU/trastuzumab seems to act antagonistically. Several of

Table 3

Outcome after standard chemotherapy for metastatic breast cancer $\pm$ trastuzumab

	AC/EC + trastuzumab	AC/EC	Paclitaxel + trastuzumab	Paclitaxel
CR	11 (8)	6 (4)	7 (8)	2 (2)
PR	69 (48)	52 (38)	31 (34)	14 (15)
Duration	9.1	6.7	10.5	4.5
Survival	26.8	21.4	22.1	18.4

Values in parentheses are in %.

the above-mentioned promising combinations were clinically tested with the combination vinorelbine/trastuzumab being particularly inviting, due to the low toxicity of both agents when given separately.

Two trials evaluated the combination of trastuzumab plus vinorelbine. The first study involved 40 patients with advanced breast cancer, the majority of which had a history of chemotherapy [85]. Trastuzumab was administered in its standard dosing regimen, patients concurrently received vinorelbine 25 mg/m² weekly. Overall response rate was 75% (8% CR, 68% PR), side effects were moderate; neutropenia was the only grade IV adverse event.

Results were reproduced in a trial with similar design including 40 previously untreated patients producing a response rate of 78% (11% CR, 67% PR) [86].

Due to the large number of chemotherapeutic agents showing activity in breast cancer, a vast number of combination regimens has been studied and have recently been the topic of several reviews [87,88].

6.4. Side effects

Trastuzumab is generally well tolerated. As with other monoclonals, infusion related reactions occur which include fever, rigors, chills, nausea, dyspnoea and hypotension. The frequency of these events is reported to be around 40% in patients receiving the first dose, with a marked reduction to <5% during the following administrations. Symptoms usually respond well to interruption of the infusion or symptomatic treatment.

An adverse event not anticipated that was first noted in the pivotal combination chemotherapy trial was cardiac toxicity. The cardiac adverse events prompted a retrospective analysis of all cases of cardiac dysfunction by an independent review and evaluation committee. Incidence of cardiac dysfunction as retrospectively found in the pivotal mono- and combination therapy trials is summarized in Table 4.

Concurrent treatment with an anthracycline, cyclophosphamide and trastuzumab significantly increased the risk of cardiac dysfunction, as compared with treatment with only an anthracycline and cyclophosphamide. A smaller increase in risk was seen after administration of paclitaxel and trastuzumab, as compared with treatment with paclitaxel alone (all these patients had previously received an anthracycline). A retrospective analysis of patients receiving trastuzumab single-agent therapy revealed a cardiac dysfunction rate of 7%.

Heart failure occurring after trastuzumab therapy generally responds well to therapy. Most patients are able to continue their treatment with the antibody.

Given the extremely poor prognosis of patients with HER2-positive metastatic breast cancer, the benefit of treatment with trastuzumab must be weighed against the risk of developing overt heart failure. For the time being, patients who are candidates for a treatment with trastuzumab should be carefully evaluated for cardiac function before and throughout therapy. Risk factors include age, pre-existing cardiac disease, a history of cardiotoxic drug treatment and radiation therapy involving the left chest. Combination of trastuzumab with anthracyclines is not recommended outside clinical trials.

7. Gemtuzumab-ozogamicin

Gemtuzumab-ozogamicin (GO) is a humanized antibody to CD33 conjugated with calicheamycin, a highly potent cytotoxic antibiotic. CD33 is expressed on leukaemic blast cells of the majority of patients with acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS). CD33 is also found on normal haematopoietic cells including erythroid, megakaryocytic and myelomonocytic progenitors; it is not expressed on pluripotent haematopoietic stem cells and can scarcely be found on non-haematopoietic tissue [89]. After

Table 4

Incidence of cardiac adverse events after trastuzumab treatment

	AC/EC + trastuzumab (N = 143) (%)	AC/EC (N = 135) (%)	Paclitaxel + trastuzumab (N = 91) (%)	Paclitaxel (N = 95) (%)	Trastuzumab (N = 213) (%)
Incidence of cardiac adverse events	28	7	11	1	7
NYHA III/IV	19	3	4	1	5

administration, the antibody portion binds to CD33, forming a complex that the target cell internalises. Following endocytosis calicheamycin is released by hydrolysis and the catalytic part of the molecule responsible for its toxicity is uncovered. Calicheamycin migrates into the nucleus producing double-strand breaks that ultimately result in cell death. In vitro studies have shown the uptake by CD33 positive cells to be 77,000 times greater than by CD33 negative cells [90]. The antibody part of the molecule can thus be regarded as a messenger that selectively transfers its toxic payload into CD33 positive target cells.

7.1. Single agent GO in relapsed AML

In a phase I study in 40 patients with refractory AML, 20% of patients achieved a reduction in peripheral blood and bone marrow blast count with normalization of peripheral blood occurring in 8% of patients [91]. Side effects were more common and pronounced than usually observed with “naked” antibodies: fever and chills occurred in 80% of patients, 20% suffered from grade 3 or 4 elevation of transaminases. Severe thrombocytopenia and neutropenia developed in the majority of patients.

Persistent thrombocytopenia was noted in some patients who otherwise achieved a complete bone marrow remission. A new category of response was proposed for GO: CR without platelet recovery (CRp) implying the established criteria for a complete response (absence of leukaemic blasts from peripheral blood, <5% blasts in bone marrow, haemoglobin level >9 g/dl, ANC > 1500 μl^{-1} , no red cell transfusion for 2 weeks, no platelet transfusion for 1 week) except the lack of a full platelet response (but with independence of platelet transfusion).

One hundred and forty-two patients (80 of them older than 60 years) with untreated first relapse of AML were subsequently enrolled in three multi-centre phase II studies [92]. Inclusion criteria included a duration of first remission of >3 months and expression of CD33 on >80% of leukaemic blasts. Patients received two doses of 9 mg/m² of GO with the second administration given 2 weeks after the first. Patients with white blood cell counts above 30,000 μl^{-1} received preceding cytorreduction with hydroxyurea to lower the count below that level.

Overall remission rate was 30%, consisting of 16% CR and 14% CRp. Median time to remission was 60 days, median length of remission was 7.2 months in patients achieving CR, and 4.4 months in patients achieving CRp.

Subgroup analysis revealed that median survival was 4.7 months in patients whose first response had lasted less than a year compared to 11.7 months in patients in remission for more than 1 year. There was no significant difference in response rate or duration on the basis of age or karyotype.

As expected, side effects were more severe than what is usually observed in standard antibody treatment [92]; compared to conventional treatment for AML, however, therapy

was very well tolerated: grade 3/4-infusion related reactions including chills, fever and hypotension were seen in one-third of patients. Grade 3 or 4 neutropenia occurred in 98%, 28% of patients developed serious infections. Severe thrombocytopenia was seen in 99% of patients, with serious bleeding occurring in 15%. Neutrophil and platelet counts typically recovered within approximately 40 days after the first administration of GO. Grade 3–4 hyperbilirubinemia was seen in 23% of patients, elevated transaminase levels in 17%. As opposed to standard chemotherapy for AML, there was a low incidence of severe nausea and vomiting (11%) and no treatment-related cardiotoxicity, cerebellar toxicity, or alopecia. Many patients received GO on an outpatient basis (38 and 41% of patients for the first and second doses, respectively). Median total duration of hospitalisation was 24 days. Based on these data, GO achieved orphan drug status and FDA approval was granted for the treatment of patients ≥ 60 years with relapsed AML not considered candidates for standard cytotoxic therapy.

7.2. GO in combination with chemotherapy

Numerous combination regimens of GO with chemotherapy for relapsed or primary refractory AML are currently undergoing testing. Some of these regimens contain cyclosporine as an inhibitor of the multi-drug resistance conveying MDR protein that is suspected to play a role in resistance to GO by increasing calicheamycin efflux from leukaemic cells [93]. First results in patients with relapsed or refractory AML show remission rates of 21% for MIA (gemtuzumab, idarubicin, cytarabine) [94], 9% for MDAC (gemtuzumab, liposomal daunorubicin, cytarabine, cyclosporine) [95], and 12% for MTA (gemtuzumab, topotecan, cytarabine) [96]. As most of the patients treated in these trials had prognostically poor AML, results can hardly be compared to single agent treatment or standard chemotherapy. Randomised trials will have to follow to establish the value of combination therapy.

7.3. GO as first-line treatment in AML

In a recent trial, 51 patients ≥ 65 years with untreated AML or advanced MDS were randomised to receive single agent GO or GO and Interleukin 11 as induction treatment [97]. A CR was achieved by 8% of patients receiving GO alone and by 36% of patients receiving GO and IL-11. In comparison with matched patients that had received standard treatment (idarubicin/cytarabine) response rates and survival were clearly inferior. The authors concluded that there is little evidence to suggest that GO with or without IL-11 should be used instead of standard chemotherapy in older patients with newly diagnosed AML or myelodysplastic syndrome.

In a phase II trial in 49 patients <65 years with de novo AML addition of one dose of 6 mg/m² of GO to standard induction treatment consisting of idarubicin/cytarabine in

newly diagnosed AML patients <60 years led to CR rate of 84% [98]. No data of randomised trials have yet been reported showing any benefit of the addition of GO to standard induction therapy.

7.4. Veno-occlusive disease after GO

Hepatic veno-occlusive disease (also called “sinusoidal obstructive syndrome”) has been reported in up to 12% of patients receiving GO [99]. The highest incidence occurs in patients undergoing treatment with combination regimens and in patients with a history of allogeneic stem cell transplantation. After treatment with single-agent GO, incidences of 1–5% are found. The purported mode of action is receptor-based uptake of the calicheamycin-antibody complex through CD33 expression on liver cells [100]. Prophylaxis with ursodiol which is reported to decrease the incidence of VOD in the setting of allogeneic stem cell transplantation does not have any effect in patients treated with GO [101]. Successful treatment with defibrotide has been described [102].

7.5. Experimental indications

Experimental indications that are currently being investigated where little or no data are yet available include the use of gemtuzumab as part of the conditioning regimen before haematopoietic stem cell transplantation, as consolidation after standard cytotoxic chemotherapy or in patients with CD33+ acute lymphatic leukaemia or chronic myeloid leukaemia in blast crisis.

8. Radio-immunotherapy

Low-grade non-Hodgkin lymphomas are exceptionally radiosensitive tumours. Patients with early stage disease can often be cured with localized radiation therapy. Unfortunately, only a small minority of patients are diagnosed early enough to allow local radiotherapy. Radio-immunotherapy (RIT) was developed to allow specific irradiation of tumour tissue with sparing of unaffected organs by coupling of radionuclides to monoclonal antibodies.

Conjugation of a monoclonal antibody and a radionuclide overcomes some of the limitations of “naked” antibodies:

- Radiation of the “hot” antibody bound to its target cell leads to a crossfire effect which can reach adjacent cells not carrying the target antigen.
- Radiation can reach cells inside a bulk not accessible to “cold” antibodies due to poor tissue penetration.
- Unconjugated antibodies require a functioning host immune system to lyse their target cell. Patients typically treated with antibodies are often immuno-compromised either by their underlying disease or as an effect of preceding therapy.

8.1. ⁹⁰Y-ibritumomab-tiuxetan

Ibritumomab-tiuxetan is a combination of the CD20-antibody ibritumomab (the murine parent molecule of rituximab) linked to tiuxetan, a chelating agent providing a high affinity binding site for radionuclides. The murine analogue was intentionally chosen as higher immunogenicity promotes rapid clearance from the circulation, limiting radiation exposure. Ibritumomab is administered with ⁹⁰Y—a beta radiator with a half-life of 64 h and a range in soft tissue of 1–5 mm—for therapeutic use.

Because of the narrow therapeutic index of radiation therapy and due to the fact that the β -rays emitted by ⁹⁰Y are completely absorbed by the patient’s body, ibritumomab labeled with ¹¹¹In—an emitter of both β - and γ -radiation—is used for imaging and dosimetry purposes before the therapeutic application: on day 1, rituximab is administered at a dose of 250 mg/m² to saturate readily available CD20 and improve biodistribution of the subsequently administered ¹¹¹In-labeled ibritumomab. Imaging studies are performed on days 1 and 3; if these show an acceptable radionuclide distribution, the therapeutic dose of ⁹⁰Y-ibritumomab is administered on day 7, again preceded by 250 mg/m² of rituximab.

The purpose of the imaging scan is to ascertain optimal biodistribution of the antibody with no vital organ or unaffected tissue receiving doses in excess of 20 Gy, a condition that was never observed in clinical trials. It is therefore possible that this precaution is modified or abandoned in the future.

The standard dose of ⁹⁰Y-ibritumomab in lymphoma patients is 0.4 mCi/kg. This corresponds to an amount of antibody of less than 5 mg, significantly less than the preinfused rituximab.

Patients with a platelet count between 100 and 150,000 ml⁻¹ receive a reduced dose of 0.3 mCi/kg. Patients with platelet counts below 100,000 μ l⁻¹ are generally not treated with ibritumomab, as are patients with a marrow infiltration of >25%, a hypocellular marrow or a history of failed stem cell mobilisation.

Due to its lack of penetrating radiation, extra shielding after therapy is unnecessary and the treatment can generally be performed on an outpatient basis. Patients are instructed to avoid exposing others to body fluids for the first few days after therapy.

⁹⁰Y-ibritumomab is generally well tolerated, the main toxicity is myelosuppression caused by radiation emitted from antibody bound to CD20 on lymphocytes and tumour cells in the bone marrow. Myelosuppression typically appears 1–2 months after therapy and lasts for 2–4 weeks. The median ANC nadir count was 800 μ l⁻¹ in clinical trials, median haemoglobin 10.3 g/dl, and median platelet nadir 37,500 μ l⁻¹.

In a randomised controlled trial, 143 patients with relapsed or refractory low-grade lymphoma were treated with either ⁹⁰Y-ibritumomab as described above or with four weekly ad-

ministrations of rituximab. Overall response rate was 80% for patients treated with ^{90}Y -ibritumomab (CR 30%), compared to 56% overall response (CR 16%) in the rituximab arm ($P = 0.002$). However, the median response duration was statistically equivalent in both arms (14.2 and 12.1 months, respectively) [103], and the increase in response rate and duration did not prolong the survival of patients treated with radiolabeled antibody as compared to standard rituximab treatment.

As an expression of its mode of action being largely independent of the intrinsic activity of the underlying antibody, in 57 patients refractory to therapy with unconjugated CD20-antibody, an overall response rate of 74% was established (15% CR, 59% PR). The estimated time to progression was 8.7 months for responders [104].

Concern has been expressed about potential long-term toxicity including the development of therapy-related myelodysplastic syndrome and the restriction of future therapeutic options due irreversible stem cell damage caused by radiation. Of 770 patients receiving ^{90}Y -ibritumomab in clinical trials and followed-up between 1.5 and 14 years, therapy related MDS or AML developed in 10, not exceptionally high in a highly pre-treated population [105]. In a retrospective study of 58 patients progressive after therapy with ^{90}Y -ibritumomab who were subsequently treated with a variety of chemotherapeutic regimens, toxicity was comparable to that of a matched control group [106].

^{90}Y -ibritumomab received orphan drug status and was FDA approved for the treatment of relapsed/refractory B-cell NHL in 2002 including patients with rituximab refractory disease. Treatment with the radiolabeled anti-CD20-antibody seems to have a somewhat higher response rate compared to treatment with “naked” antibody, this does not however lead to increase survival however. Toxicity (i.e. reversible myelosuppression) is considerable compared with rituximab and there is ongoing concern of long-term safety of radiation exposure. Impressive response rates in patients refractory to rituximab indicate that this setting might be the ideal indication for radioimmunotherapy.

8.2. ^{131}I -tositumomab

Another radioimmunotherapeutic agent targeting the CD20 antigen is ^{131}I -labeled tositumomab. ^{131}I -tositumomab has several similar properties to ^{90}Y -ibritumomab and others that distinguish it (see Table 5).

Due to the many years of experience with the isotope in the treatment of thyroid cancer, ^{131}I is a relatively inexpensive and readily available pharmaceutical agent. Because of highly variable elimination of ^{131}I among patients, dosimetry is necessary to calculate each patient's individual dose, with a target total body radiation dose of 75 cGy. The nature of ^{131}I as a combined β - and γ -emitter with the γ -radiation penetrating tissue up to one metre allows dosimetry by administration of the therapeutic drug. The downside of this effect is that the total body dose delivered to the patients by

Table 5
Comparison of ^{90}Y -ibritumomab and ^{131}I -tositumomab

	^{90}Y -ibritumomab	^{131}I -tositumomab
Monoclonal antibody	Murine (ibritumomab)	Murine (tositumomab)
Preinfusion antibody	Chimeric (rituximab)	Murine (tositumomab)
Conjugation	Chelation	Direct iodination
Isotope	^{90}Y	^{131}I
Isotope half-life	64h	8d
Radiation emitted	β	$\beta\gamma$
Imaging possible	No	Yes
Beta energy (MeV)	2.3	0.8
Path length (mm)	5.3	0.8

the drug is higher than by its ^{90}Y -carrying counterpart ^{90}Y -ibritumomab. Patients receiving the drug should avoid close contact with other people, sleep alone and keep away from public places 3–4 days after drug administration due to their emission of radiation. Dehalogenation, i.e. cleavage of the radionuclide from the antibody, can lead to incorporation of ^{131}I into the thyroid gland with subsequent hypothyroidism, although this is usually reduced by the pre-treatment administration of iodine solutions. Similar to ^{90}Y -ibritumomab, ^{131}I -tositumomab administration is preceded by an infusion of unlabeled antibody, in this case “cold” tositumomab.

Clinical trials with ^{131}I -tositumomab preceded those with ^{90}Y -ibritumomab, longer follow-up and more reliable information of long-term toxicity is therefore available. Similar patient populations have been treated with ^{131}I -tositumomab, and results of non-myeloablative RIT with the drug are comparable to what is achieved with ^{90}Y -ibritumomab: in a trial with 59 patients with relapsed or refractory advanced lymphoma an overall response rate of 71% was achieved. Median survival of responders was 12 months [107]. The response rate in patients progressing after therapy with rituximab is only slightly lower with 68% [108].

In an interesting observation, median dose delivered to tumours was 795 cGy, more than 10 times the mean total body dose [109]. As myelotoxicity is the main dose-limiting side effect of radio-immunotherapy, the concept of myeloablative therapy with stem cell rescue was investigated already early in the development of the drug. In a trial enrolling 24 patients with relapsed low-grade NHL, a myeloablative dose of ^{131}I -tositumomab was administered. Apart from the expected marrow toxicity, effects on other organs were acceptable and considerably lower than in conventional myeloablative therapy [110]. Dose-limiting toxicity was cardiopulmonary insufficiency seen in patients receiving pulmonary doses in excess of 27 Gy. Overall response was 87% with a CR rate of 81%. In an update, 5-year survival rate was reported to be 70% [111].

In a retrospective analysis of 125 patients receiving autologous stem cell transplants after either myeloablative RIT or a combination of total body irradiation and high-dose chemotherapy, 5-year overall survival was significantly higher in the RIT group (67% versus 53%) [112]. Regimens

incorporating RIT as a replacement for total body irradiation are being explored.

In 2003, ^{131}I -tositumomab achieved FDA approval for the non-myeloablative treatment of patients with B-cell NHL refractory to rituximab.

9. New compounds and outlook

After initial enthusiasm following the first results of monoclonal antibodies in the treatment of cancer and more than a decade of disappointment due to the failure to reproduce the success achieved in single patients on a larger scale, MABs have finally found their way into the routine armamentarium of the clinical oncologist. Still, the vast majority of molecules that has undergone testing have failed to deliver clinical benefits. Despite impressive advances leading to the production of engineered molecules with increased serum half-life and the ability to recruit effector cells, developing effective treatments against common solid tumours such as breast, lung and colon cancer has proven exceedingly difficult. Reasons for the inferiority of results in solid tumours compared to the treatment of haematological malignancy include

- the increased interstitial pressure found in solid tumours compromising penetration by high molecular weight compounds;
- production of tight intracellular adhesion-molecules;
- secretion of proteoglycan molecules binding antibody molecules unspecifically;
- the absence of effector cells inside the tumour.

One way to overcome these obstacles lies in patient selection. The first patients typically receiving newly developed oncological drugs are patients with advanced disease that are refractory to or have relapsed after standard treatment. However, experience from patients treated with currently available antibodies shows that this treatment modality might be most effective in the minimal residual disease setting.

Another reason why early trials with monoclonal antibodies mostly failed, was that the significance of the target molecule was under-appreciated. The notion that an optimal target for antibody therapy is one that is exclusively expressed on cancer and that coating the malignant cell with antibody will invariably lead to clinical response had to be revised. Of the antibodies discussed above, one targets a growth factor receptors (trastuzumab), another one induces apoptosis by cross-linkage and calcium influx (rituximab) and one targets CD52, making cells exceptionally susceptible to complement mediated lysis by mechanisms that are not yet understood [113]. The remaining molecules (gemtuzumab-ozogamicin, ibritumomab-tiuxetan and tositumomab) rely on their toxic payload to destroy their target cells.

The list of antibodies recently entering routine clinical use or undergoing advanced phase clinical testing reflects the insight that the physiological function of the target is crucial for the success of antibody therapy and that for tu-

mours where no ideal target is found, additional arming of the molecule by means of radiolabeling, conjugation with a cytotoxic molecule or alternative techniques are necessary.

Two compounds that recently received FDA approval are cetuximab and bevacizumab, both targeting growth factor receptors. Reflecting the increased efficacy seen in the currently approved antibodies when used in combined modality treatment, both agents have already early in their development been tested as an addition to standard chemotherapy.

9.1. Cetuximab

Cetuximab (Erbix[®]) is a chimeric monoclonal antibody against epidermal growth factor receptor (EGFR), expressed on a variety of malignant tumours such as non-small-cell lung cancer, head and neck or colon cancer.

In patients with metastasised colon cancer refractory to treatment with irinotecan, single agent cetuximab showed a response rate of 10.8% with a median TTP of 1.5 months. Combination with irinotecan increased the response rate to 22.9% and median TTP to 4.1 months [114]. Based on these data, cetuximab received FDA approval in 2004 for the treatment of metastasised colorectal cancer in combination with irinotecan in patients refractory to irinotecan chemotherapy or as single agent treatment in patients intolerant to irinotecan-based chemotherapy.

Other indications are currently undergoing testing. One of the most promising is non-small-cell lung cancer where the addition of cetuximab to cisplatin/vinorelbine in inoperable patients increased response rates from 29 to 50% in a small randomised trial [115].

Cetuximab has also been extensively investigated in patients with head and neck cancer (HNC). A Phase III study recruited 121 patients with untreated HNC metastatic disease to compare cisplatin and cetuximab combination therapy to cisplatin alone. The response rate was higher in the combination treatment group: 23 versus 9%. However, there was no significant difference in time to tumour progression (4.1 and 3.4 months, respectively) or overall median survival (9.2 and 8.0 months, respectively [116]).

9.2. Bevacizumab

The addition of bevacizumab (Avastin[®])—a humanized antibody against vascular endothelial growth factor (VEGF)—to irinotecan/5-FU/leucovorin was tested in a randomised trial as first-line treatment in patients with metastatic colon cancer. Addition of the antibody increased overall response rate from 35 to 45% and prolonged median survival from 15.6 to 20.3 months [117]. Based on these data, bevacizumab received FDA-approval for first-line treatment of metastasised colorectal cancer in combination with a 5-FU based treatment in 2004.

Another indication where Bevacizumab has recently been tested is metastatic renal cancer, a tumour with high expression of VEGF where bevacizumab was able to prolong me-

dian TTP by a factor of 2.5 compared to placebo [118]. No effect on survival was seen however. Similar results were seen in a Phase III study in patients with metastasised breast cancer, where the combination of capecitabine and bevacizumab was compared to treatment with capecitabine alone. Overall response rate rose significantly from 19 to 30%, but this did not lead to increased progression free or overall survival [119]. It will be interesting to see whether using bevacizumab earlier in the course of the disease—as is currently being investigated—will provide more clinical benefit. Other indications where bevacizumab is being tested in early stages include prostate, pancreas and non-small-cell lung cancer. No randomised data are yet available of the possible benefit in these settings.

Targeting tumor vasculature has become an increasingly attractive approach. Advantages of this approach include its applicability to various tumour types, its low toxicity on normal tissue, the low likelihood of resistance due to the genetic stability of antigen expression on endothelial cells which are not themselves transformed, and the good accessibility of the target structure to the antibody. Besides VEGF, targets for monoclonal antibody based anti-angiogenic therapies include the basic fibroblast growth factor, several other growth factor receptors and a number of cell adhesion molecules [120].

Dozens of other MAbs are currently being tested in pre-clinical and clinical trials. It would be beyond the scope of this review to provide a comprehensive list. An extensive description of molecules currently undergoing advanced phase clinical trials has recently been published elsewhere [121].

9.3. Increasing antibody effector functions

Another approach to increase the clinical efficacy of anti-cancer antibodies lies in engineering available antibodies to maximize effector functions. Areas of research include

- engineering the Fc part of the antibody to increase binding by Fcγ-receptors and thereby maximize ADCC;
- fusion of antibodies with cytokines;
- attachment of antibodies to liposomes containing drugs, toxins or DNA for gene therapy, overcoming the need to use extremely toxic substances such as calicheamycin that can be directly bound to the antibody;
- production of bispecific antibodies, molecules which bind to a tumour-associated antigen with one arm, while the other arm binds to a so-called trigger antigen, such as Fc-receptors or CD3 allowing increased ADCC with the recruitment of additional cells such as T-lymphocytes;
- antibody-dependent enzyme-mediated prodrug therapy (ADEPT) involving an antibody-enzyme fusion protein administered in a first step and allowed to localize to the tumor, followed by a prodrug administered after the remaining fusion protein has cleared.

Several excellent reviews recently covered these emerging technologies [122,123], which have shown exciting results in

animal models and early clinical studies. More than 25% of all pharmacological agents currently under development are antibody-based [122,123], and considering the pace of development and the frequency of antibodies achieving FDA-approval in the last few years, medical oncology can expect a broad extension of its armamentarium in the near future.

Reviewer

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Biography

Professor Richard Herrmann received his medical training at the University of Heidelberg, Germany. From

1977–1979, he held a Clinical Fellowship in Medical Oncology at the Roswell Park Cancer Center Institute in Buffalo, USA. On his return, he was appointed assistant professor at the University of Heidelberg, before moving on to the Free University of Berlin in 1985 and then to his current position of chief of medical oncology at the University of Basel in 1991. In 1985, Professor Herrmann received the Award of the German Medical Oncology Association. His research interests include cancers of the gastrointestinal tract, lymphomas and phase I/II studies with innovative approaches. He is President of the Swiss Group for Clinical Cancer Research (SAKK) and associate editor of *Oncology*.