Myeloablative radioimmunotherapy in conditioning prior to haematological stem cell transplantation: closing the gap between benefit and toxicity?

Inga Buchmann · Ralf G. Meyer · Walter Mier · Uwe Haberkorn

Abstract High-dose radio-/chemotherapy in the context of autologous and allogeneic haematopoietic stem cell transplantation is a double-edged sword. The requirement for dose intensification is linked to an increase in toxicity to noninvolved organs. Particularly for older patients and patients with comorbidities, efficient but toxicity-reduced schemes are needed. Myeloablative radioimmunotherapy is a targeted, internal radiotherapy that uses radiolabelled monoclonal antibodies (mAb) with affinity to the bone marrow. It involves the administration of high radiation doses (up to 30 Gy) to the bone marrow and spleen but without exposing radiosensitive organs to doses higher than 1–7 Gy. Added to conventional or intensity-reduced conditioning, myeloablative radioimmunotherapy may achieve a pronounced antileukaemic effect with tolerable toxicities. A rational and individual design of the ideal nuclide–antibody combination optimizes therapy. The anti-CD33, anti-CD45 and anti-CD66 mAbs appear to be ideal tracers so far. The β-emitter ⁹⁰Y is coupled by DTPA and is the best nuclide for myeloablation. Approval trials for DTPA anti-CD66 mAb are underway in Europe, and in the near future these therapies may become applicable in practice. This review gives an overview of current myeloablative conditioning radioimmunotherapy. We discuss the selection of the optimal radioimmunoconjugate and discuss how radioimmunotherapy might be optimized in the future by individualization of therapy protocols. We also highlight the potential advantages of combination therapies.

Keywords Leukaemia · Multiple myeloma · Myeloablative conditioning · Myeloablative radioimmunotherapy · Stem cell transplantation

Introduction

Intensification of the dose of chemo- and/or radiotherapy is the main therapeutic principle of autologous stem cell transplantation for haematological diseases such as multiple myeloma (MM). In allogeneic stem cell transplantation for diseases such as myelodysplastic syndromes (MDS) or acute leukaemia, high-dose chemotherapy also involves the eradication of the bone marrow and immunosuppression to facilitate the establishment of donor haematopoiesis. While intensification of the therapy doses reduces the probability of relapse on the one hand, it increases therapy-related toxicity and mortality on the other. However, even with intensified chemo- and radiotherapy, a significant proportion of patients with high-risk disease do not achieve long-term disease-free survival after stem cell transplantation. Further intensification of conditioning therapies might therefore help to reduce relapse rates, although it is associated with an increase in therapy-related morbidity. In addition, in aggressively pretreated or elderly patients (such as those with MM), comorbidities and organ dysfunctions often limit the applicability of highly aggressive conditioning therapies which may result in a negative impact on long-term disease control [1, 2].
For conditioning prior to stem cell transplantation, internal, targeted radiation therapies using radiolabelled antibodies with affinity to haematopoietic or bone marrow stromal cells are able to specifically deliver high radiation doses to the bone marrow. The adjacent tissues and organs are sufficiently spared, and toxicities can thereby be limited. The β-emitter ⁹⁰Y is most suited for myeloablative radioimmunotherapy, and anti-CD33, anti-CD45 and anti-CD66 antibodies are ideal tracers. In theory, this targeted radiotherapy therefore combines intensification of the radiation dose with protection of uninvolved tissues.

In this review, we provide an overview of radioimmunotherapeutic principles, followed by a critical evaluation of the potential and limitations of radioimmunotherapy for myeloablation in combination with conventional conditioning schemes. We discuss how to select the ideal radioimmunoconjugate(s) in relation to the kind of disease, the form of transplantation, the remission status and the presence of extramedullary tumour cells. The focus is the treatment of acute myeloid leukaemia (AML), MDS and MM prior to allogeneic as well as autologous stem cell transplantation. Although only a few clinical trials have been reported for myeloablative radioimmunotherapy of chronic myeloid leukaemia (CML) so far, the outcome in patients with CML after stem cell transplantation highly depends on graft-versus-leukaemia effects rather then on intensification of conditioning therapy and is, therefore, not covered in this article. Radioimmunotheories for the treatment of malignant lymphoma are also not focused on in this review.

Principles of radioimmunotherapy

Radioimmunotheories are internal, targeted radiation therapies that deliver high radiation doses to the tumour while sparing noninvolved tissues and organs. They depend on the specific binding of the radiolabelled antibody to an antigen which is overexpressed in the target tissue. Which antibody is the most adequate depends on its binding specificity, biodistribution and biokinetics.

The optimal radionuclide is determined by physical, chemical and biological characteristics. Most commonly, β⁻ particles are used. They target tumour cells in a variable diameter of up to 10 mm around the bound cell which is described by the term “cross-fire effect”. Nuclides with a high tissue range have a high cross-fire effect. They induce a high and homogeneous energy in the target tissue and are well suited to inducing myeloablation or to the treatment of large tumour bulks. In contrast, the cross-fire effect of β⁻ emitting nuclides with a low tissue range and especially that of alpha-particle emitters is lower. Targeted therapies with these nuclides offer the potential for more specific target cell killing with low damage to surrounding normal tissues. They facilitate a high energy transfer for the treatment of small residual tumour masses and single tumour cells or at the margins of a larger target tissue, but are less suited to myeloablation [3, 4].

Clinical background

Acute leukaemia, myelodysplasia and multiple myeloma

AML and acute lymphoblastic leukaemia (ALL) are neoplastic diseases of the early haematopoietic stem cells. In adult ALL, allogeneic haematopoietic stem cell transplantation is considered in the presence of unfavourable parameters such as the presence of certain cytogenetic changes (e.g. t(4;11), t(9;22)), late achievement of first remission, and high cell counts at first diagnosis. The relapse rate after allogeneic stem cell transplantation is still in the range 25–30% for patients in first remission and up to 50% in second remission [5]. Autologous transplantation does not result in higher efficiency than conventional chemotherapy [6]. Older age (>35 years) is associated with an increase in treatment-related mortality mainly caused by the toxicity of the conditioning regimen and by infections [6]. The still high relapse rate indicates the need for dose intensification of the conditioning regimen especially in patients in second remission.

AML is the most common adult leukaemia. With initial chemotherapy, remission is induced in the majority of patients and the intensity of subsequent consolidation therapy depends on the individual risk. This risk is mainly related to cytogenetic findings and molecular markers. In the absence of a favourable karyotype [7] depending on additional molecular markers [8, 9] allogeneic stem cell transplantation is the treatment of choice. This is also the case in every patient with second remission after initial relapse. The radiation sensitivity of AML has led to trials increasing the dose of total body irradiation from 12 Gy to 15.75 Gy. This increase is able to reduce the relapse rate but does not lead to improved survival due to an increase in toxicity [10]. These findings in particular argue in favour of the concept of targeted radiotherapy.

Myelodysplasia, also a clonal stem cell disorder, is a heterogeneous group of diseases with regard to the clinical picture and the risk of advance to AML. The new WHO classification [11] has advanced the FAB classification by including cytogenetic findings as well as adjusting the cut-off value for the blast infiltration of the bone marrow between refractory anaemia with excess of blasts (RAEB) and AML from 30% to 20%. Allogeneic stem cell transplantation is considered depending on the International Prognostic Scoring System (IPSS) that includes cytogenetics, blast count in the bone marrow, and the number of affected haematopoietic lineages. However, since MDS is a disease of the older
patient and age as a major risk factor for allogeneic stem cell transplantation, intensive conditioning regimens are often contraindicated. Reduced intensity conditioning in older patients has been proven feasible [12] but is still associated with an increased risk of relapse compared to dose-intensive regimen [13]. Targeted radioimmunotherapy might be able to close this gap in the future.

MM is one of the most common haematological malignancies with an incidence of 4/100,000 and autologous stem cell transplantation is part of the standard treatment for patients up to the age of 65 years [14, 15], but not all studies have shown survival benefit for this approach [16]. In recent years and in the context of new therapeutic options, however, the role of autologous stem cell transplantation might have to be redefined. Still, neither conventional chemotherapy and new drugs nor autologous transplantation can cure MM. Only allogeneic stem cell transplantation offers a curative potential for a small number of patients at the price of a high treatment-related mortality. Therefore, the use of allogeneic stem cell transplantation for MM is a matter of debate and it should only be used within the context of clinical trials. MM is a disease of intermediate radiosensitivity and regimes including total body irradiation for conditioning are inferior to high-dose melphalan.

However, since the clonogenic progenitor cell of the myeloma is relatively resistant to chemotherapy, it is likely to be the major source of relapse [17].

Stem cell transplantation

Haematopoietic stem cells can be used to recover haemopoiesis after intensified chemotherapy in many haematopoietic diseases. In principle, haematopoietic stem cells can be obtained directly from the bone marrow or from the peripheral blood following drug-induced mobilization from the bone marrow. The radio-chemotherapy used prior to stem cell transplantation is called conditioning. In autologous stem cell transplantation the conditioning regimen is mainly used to treat the underlying malignant disease. By the readministration of autologous stem cells that have been collected and cryopreserved prior to the dose-intensive radio-/chemotherapy, the dose-limiting toxicity to the patient’s bone marrow can be overcome (dose escalation). In the treatment of haematological malignancies, autologous stem cell transplantation is in general reserved for more moderate disease (such as some lymphomas with a bad prognosis or relapsing). In contrast, allogeneic stem cell transplantation is mainly reserved for high-risk diseases such as acute leukaemia and some high-risk or relapsed MDS. The conditioning regimen prior to allogeneic transplantation also eradicates the patient’s (host’s) haemopoiesis to make room for the donor stem cells and suppresses the host’s immunity to prevent graft rejection by host-derived T cells.

The most frequently used high-dose chemotherapy for allogeneic transplantation is 60 mg cyclophosphamide per kilogram body weight per day for 2 days in combination with further conditional treatment, such as busulfan [18] or fractionated total body irradiation.

Compared to autologous transplantations, allogeneic transplants induce immunological reactions. The graft-versus-tumour effects account for reduced rates of relapse [19]. The graft-versus-host disease (GvHD), however, contributes to higher toxicity and therapy-related mortality [20]. TNF-α has been discussed as a relevant mediator of acute GvHD if its levels are increased during aggressive, conventional conditioning schemes [20–22].

The toxic effects of myeloablative allotransplantation increase with the age, particularly after the age of 50 years, and generally preclude performing the procedure in patients older than 65 years. For older recipients with comorbidities and organ dysfunction, reduced-intensity regimens are needed. The low mortality rate associated with reduced-intensity preparative regimens may, however, be offset by a high relapse rate [23].

The outcomes after stem cell transplantations vary depending on the type and stage of disease, the age and functional level of the patient, the source of the stem cells to be transplanted and the degree of the HLA mismatch [19]. The 5-year disease-free survival of patients with acute leukaemia after stem cell transplantation has been reported to vary from 15% to 65% after allogeneic transplantation and from 5% to 50% after autologous transplantation [19]. Intensity-reduced conditioning may result in a relapse-related 5-year mortality rate of 54%. In contrast, highly aggressive conditioning regimens may be associated with a therapy-related mortality as high as 64% [24–27]. Although the therapy-related mortality rate is less than 10% for some allogeneic transplantations, about 40% of patients with advanced disease who undergo allogeneic transplantation usually die from complications related to the transplantation [19].

All these findings show that conditioning therapies are required that increase the toxic bone marrow effects without significantly increasing severe extramedullary toxicities. Internal, targeted irradiation with radiolabelled antibodies that accumulate in the haematopoietic marrow fulfil these requirements.

Myeloablative radioimmunotherapy schemes

Doses of 2–8 Gy applied to the bone marrow induce moderate myelosuppression, and doses above 8–10 Gy induce relevant hypoplasia or aplasia of the bone marrow and require subsequent stem cell transplantation. Myelo-
Blation as part of radioimmunotherapy has two different aims (Table 1):

**Myeloablation as the major therapy aim**

If radioimmunotherapy is used in the context of a conditioning regimen prior to stem cell transplantations it might comprise both myeloablation and reduction in the medullary tumour load. The radioactive antibodies bind to cells of the normal haematopoietic bone marrow and operate by the cross-fire effect that covers the medullary compartment. Additional specific binding to the malignant cells is necessary for two reasons: (1) the bone marrow is highly infiltrated by leukaemic blasts and antigen-expressing normal haematopoiesis is largely replaced, or (2) extramedullary tumour cells are also present.

Myeloablative radioimmunotherapy is usually performed between 10 and 14 days prior to the transplantation before the conventional or intensity-reduced conditioning is applied [28, 29].

**Myeloablation as a toxic, unwanted side effect**

As well as part of conditioning protocols, radioimmunotherapy is also used in the treatment of haematological malignancies such as non-Hodgkin lymphoma and also of solid tumours. Unwanted myeloablation by radioimmunotherapy may occur as a side effect even if the tumours are mainly located outside the bone marrow. The radiolabelled antibodies accumulate in the bone marrow even if only some tumour cells are infiltrating the bone marrow or if the targeted antigen is not selective for the malignant cells but is also expressed on healthy haematopoietic cells. In these cases, autologous stem cell support is required to limit severe therapy-induced cytopenia and thereby prevent life-threatening complications such as severe infections caused by persistent immunosuppression [30–32].

**Dosimetry in myeloablative conditioning radioimmunotherapy**

The dose applied to the bone marrow is limited to 35–40 Gy in order to avoid stroma cell damage that may affect engraftment of the transplanted stem cells [28]. However, the maximum tolerated dose (MTD) for the bone marrow has yet to be defined. For liver and kidneys, upper limits have been defined at 10–12 Gy [29, 33]. The radioimmunoconjugates that have currently been used for myeloablative conditioning do not result in critical lung doses.

To calculate the specific organ doses an individual dosimetry has to be performed for at least 2 days. The protocols are variable but mostly include two whole-body scintigraphy scans and measurements of the radioactivity per day in the collected urine and single examinations on the following days. For each scintigraphy scan, regions of interest are drawn around the whole body, liver, kidneys, spleen and lungs, and, as examples of the bone marrow, around vertebral body L4 or the sacrum, and the estimated radiation absorbed doses are usually calculated using the MIRD scheme [34]. The precise microdosimetric estimation of the haematopoietic marrow dose still remains an unresolved topic, and the degree of miscalculation is considered to be in a relevant range.

**Radioactive nuclides**

**α-Particles**

α-Particles have a short path-length of 50–100 μm and a high linear energy transfer (approximately 100 keV/μm). For the safe use of α-particles, antibodies with very high tumour cell specificities and affinities are required, and the radiolabelling has to be highly stable in vivo. Dissociation

---

**Table 1** Principals of myeloablative radioimmunotherapy dose schemes

<table>
<thead>
<tr>
<th>Aim of therapy</th>
<th>Myeloablation as toxic effect</th>
<th>Myeloablation as major aim</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody specification</td>
<td>Reduction of tumour cell number</td>
<td>Conditioning prior to stem cell transplantation by reduction of tumour cell number, and myeloablation</td>
</tr>
<tr>
<td>Applied dose (Gy)</td>
<td>Tumour cell affinity</td>
<td>Bone marrow affinity (Tumour cell affinity)</td>
</tr>
<tr>
<td>Haematological toxicity</td>
<td>&gt;8–10</td>
<td>&gt;8–10</td>
</tr>
<tr>
<td>Stem cell transplantation</td>
<td>Severe, ablative (toxic side effect, unwanted)</td>
<td>Severe, ablative (major therapy aim, wanted)</td>
</tr>
<tr>
<td>Examples of targeted tumours</td>
<td>Yes, autologous, as &quot;stem cell rescue&quot;</td>
<td>Yes, allogeneic or autologous, as main therapy</td>
</tr>
<tr>
<td></td>
<td>High-dose radioimmunotherapy of solid tumours, or non-Hodgkin lymphoma with autologous &quot;stem cell rescue&quot;</td>
<td>Acute leukaemia prior to allogeneic stem cell transplantation</td>
</tr>
</tbody>
</table>
of the antibody and nonspecific binding of the radio-immunoconjugate may induce relevant side effects [3].

As a result of their low tissue range, inappropriate half-life (Table 2) and very restricted availability, α-particle emitters such as $^{213}$Bi and $^{225}$Ac are only sporadically used in clinical trials. They are not sufficiently myeloablative to be used in conditioning prior to haematopoietic stem cell transplantation.

$\beta^-$-Emitting nuclides

$\beta^-$-Emitting nuclides emit electrons. $\beta^-$ particles with higher tissue ranges of up to 10 mm are ideal both for the treatment of larger tumour cell conglomerates and for myeloablative conditioning before stem cell transplantation. $\beta^-$ particles with lower tissue ranges are preferred for the treatment of minimal residual disease and marginal tumour cells.

$^{90}$Yttrium $^{90}$Y is a generator nuclide that is commercially available. It has a high cross-fire effect and is one of the most suitable $\beta^-$-emitter for use in clinical trials of myeloablative conditioning so far. As a pure $\beta^-$-emitter, the nonspecific irradiation exposure of patients, medical staff and family members is low, which allows use in outpatient protocols. The half-life (Table 2) is short enough to transfer the patient from the nuclear medicine ward to the transplantation ward before the hypoplasia/aplasia becomes clinically relevant. In addition, it does not compromise the haematopoietic engraftment. $^{90}$Y is stably conjugated to the antibody by chelators derived from DTPA (diethylenetriaminepentaacetic acid) or DOTA (1,4,7,10-tetraazacyclododecane-tetra-acetic acid). The formation of DOTA complexes is a slow reaction and the use of the acyclic chelators derived from DTPA is therefore favoured for the labelling of peptides. The backbone substituted derivates of the chelator DTPA provide a higher complex stability than DTPA and can therefore be used for in-vivo applications. Several methyl-substituted isomers of DTPA have been studied. The most common derivatives are ITC-CHX-DTPA [2-(4-isothiocyanatobenzyl)-diethylenetriaminepentaacetic acid] and ITC-1B4M-DTPA [2-(4-isothiocyanatobenzyl)-3-methyl-diethylenetriamine pentaacetic acid]. CHX-DTPA has been shown to be less suitable than ITC-1B4M-DTPA (MX-DTPA) with regard to serum stability and biodistribution [35].

Because $^{90}$Y is a pure $\beta^-$-emitter, it is not suitable for dosimetry. Exact data can be obtained with positron emission tomography with $^{86}$Y. $^{86}$Y is chemically identical to $^{90}$Y, but is expensive, has a restricted availability, induces a high radiation exposure of the patient and medical staff, and requires complex measurement techniques [34]. It is not practicable in clinical routine. Therefore, $^{111}$In is most frequently used for dosimetry prior to $^{90}$Y therapy. It emits $\gamma$-quants of 171 and 245 keV and can be imaged by a $\gamma$-camera.

$^{131}$Iodide $^{131}$I is a commercially available reactor nuclide. It emits both, $\beta^-$ and $\gamma$ radiation (predominantly 364 keV) (Table 2). It is efficient for dosimetry as well as for therapy. The cross-fire effect is low, and the long half-life of 8 days of the $\gamma$ component necessitates isolation of the patient on the nuclear medicine ward for 1–2 weeks. Free $^{131}$I accumulates in the thyroid which has to be blocked by prophylactic oral administration of sodium perchlorate. Radiolabelling procedures are simple. Since labelling techniques for $^{90}$Y have been established, $^{131}$I is rarely used for myeloablative conditioning.

$^{188}$Rhenium $^{188}$Re is a generator nuclide with limited commercial availability ($^{188}$Wg/$^{188}$Re-generator; Oak Ridge National Laboratory, Oak Ridge, TN). It emits both, $\beta^-$ and $\gamma$ radiation (155 keV) and can be used for dosimetry and therapy. Radiolabelling of the antibody is carried out by complex formation with disulphide bridges. The cross-fire effect and its half-life (Table 2) are sufficient for myeloablative schemes. Free, circulating perrhenate accumulates in the thyroid, and thyroid blockade with sodium perchlorate is essential.

The most serious disadvantages in comparison with $^{90}$Y-labelled antibodies are the significantly higher nephrotoxicity of $^{188}$Re-labelled antibodies and the restricted availability [36].

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Radiation</th>
<th>Physical half-life</th>
<th>Particle energy, maximum/mean (MeV)</th>
<th>Tissue range of particles, maximum/mean (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{131}$I</td>
<td>$\beta^-, \gamma$</td>
<td>8 days</td>
<td>0.6/0.19</td>
<td>2.1/0.4</td>
</tr>
<tr>
<td>$^{177}$Lu</td>
<td>$\beta^-, \gamma$</td>
<td>6.7 days</td>
<td>0.5/0.15</td>
<td>1.6/0.3</td>
</tr>
<tr>
<td>$^{188}$Re</td>
<td>$\beta^-, \gamma$</td>
<td>17 h</td>
<td>2.1/0.8</td>
<td>10/3.1</td>
</tr>
<tr>
<td>$^{90}$Y</td>
<td>$\beta^-$</td>
<td>2.7 days</td>
<td>2.3/0.9</td>
<td>11/3.8</td>
</tr>
<tr>
<td>$^{225}$Ac</td>
<td>$\alpha$</td>
<td>10 days</td>
<td>5.8 and 8.4</td>
<td>0.04–0.08</td>
</tr>
<tr>
<td>$^{213}$Bi</td>
<td>$\alpha$</td>
<td>46 min</td>
<td>5.9</td>
<td>0.05–0.07</td>
</tr>
</tbody>
</table>

* Only particle energies that are relevant for therapy are mentioned.
**177Lutetium** 177Lu is a commercially available reactor nuclide. It is conjugated to the mAb by the same chelators as 90Y. 177Lu is a $\beta^-$ emitter with a $\gamma$ component of 208 keV in 11% of decays. It is suitable for both dosimetry and therapy. The particle energy, tissue range and the cross-fire effect are considerably lower than those of 90Y and 188Re but are more similar to those of 131I. Compared with 131I, the conjugation to the antibody is more stable and the half-life is shorter. 177Lu is suitable for the treatment of small tumour conglomerates, extramedullary minimal residual disease and marginal medullary tumour cells. It has not been used in the conditioning of patients prior to stem cell transplantation so far, but is well established in internal radiotherapies of neuroendocrine tumours [37].

**Antibodies**

In actual clinical trials, antibodies with specificity for CD33, CD45 and CD66 are used for myeloablative conditioning of patients with AML, MDS or MM prior to stem cell transplantation. All antibodies bind to normal haematopoietic cells. Anti-CD66 mAb [38] binds to leukaemic blasts to a lower proportion than anti-CD33 and anti-CD45 mAb.

**Anti-CD33 antibodies** CD33 is expressed from the pro-myelocyte stage to the stage of mature myeloid cells and most AML blasts. It is classically not expressed on ALL-B and ALL-T blasts. Currently, two different anti-CD33-antibodies have been used in clinical trials. The murine antibody M195 [39] (isotype IgG2a) and the humanized antibody HuM195 [40, 41]. They are not approved for myeloablative radioimmunotherapy.

**Anti-CD45 antibodies** The CD45 antigen is expressed on almost all leucocytes with the exception of plasma cells and is also expressed on 80–95% of all leukaemic blasts of the myeloid and lymphoid line and on the clonogenic myeloma “stem” cell [17]. For myeloablative conditioning, the murine antibody BC8 (isotype IgG1) [28, 42], the rat antibody YTH24.5 (isotype IgG2b) [43] and the rat antibody YAML568 (isotype IgG2a) [44] have been studied in clinical trials. None of these antibodies is approved for radioimmunotherapy.

**Anti-CD66 antibodies** CD66 antigens belong to the group of NCA (nonspecific cross-reacting antigens). CD66b is expressed from the promyelocyte stage to the mature granulocyte stage, but rarely on myeloid leukaemic blasts. The murine anti-CD66b mAb BW250/183 (isotype IgG1; NCA 95) is commercially available (Scintimun, Besilesomab; CIS Bio International, Schering, Switzerland) and is approved for diagnostic 99mTc scintigraphy for the imaging of infection, but not for radioimmunotherapy.

**Clinical radioimmunotherapy trials**

The feasibility, applied organ doses, toxicities and primary response data of myeloablative radioimmunotherapy have already been established in clinical conditioning studies (Table 3), and the specific organ doses and the ratios of marrow/organ doses of some selected 188Re and 90Y radioimmunoconjugates are presented in Table 4. Randomized studies comparing the efficiency of conditioning schemes with or without myeloablative radioimmunotherapy, however, have not been performed so far. Comparison with historical groups is mainly inadequate because the transplantation protocols among studies as well as the patients’ therapeutic histories are diverse.

**Conditioning of patients with AML and MDS**

*Therapies with radiolabelled anti-CD33 mAb* Jurcic et al. reported a study of 30 patients (16 with refractory or
relapsed AML; 14 with CML in relapse or accelerated phase) treated with 4.4–14 GBq $^{131}$I-M195 (19 patients) or $^{131}$I-HuM195 (11 patients). Additional conditioning included cyclophosphamide 90–120 mg/kg body weight and busulfan 16 mg/kg. All patients received allogeneic stem cells. Complete remission was seen in 93% of patients. After a follow-up period of 4.5–8 years, 19% of patients with AML were alive and disease-free. Toxicities were not significant and engraftment was in the normal range in all patients [51].

The first proof-of-concept for internal targeted α-particle immunotherapy in humans was performed in non-myeloablative doses. Jurcic et al. reported another study of 18 patients with relapsed or refractory AML or chronic myelomonocytic leukaemia treated with 10.4 to 37.0 MBq/kg $^{213}$Bi-HuM195. In 14 patients (78%), the percentage of bone marrow blasts was reduced. Extramedullary toxicity and myelosuppression were moderate, and stem cell transplantation was not necessary [52]. α-Particle radioimmunotherapy has not been used for myeloablative conditioning in leukaemia patients so far.

**Therapies with radiolabelled anti-CD45 mAb** Matthews et al. reported a study of 44 patients with advanced acute leukaemia or MDS in a remission stage after first complete remission treated with 2.8–22.6 GBq $^{213}$I-anti-CD45 mAb BC8 prior to allogeneic or autologous stem cell transplantation in combination with cyclophosphamide 120 mg/kg body weight and fractionated total body irradiation (12 Gy) [42]. Marrow doses obtained with radioimmunotherapy were up to 30 Gy and maximum liver doses were 7 Gy [28, 42]. During a follow-up period of 65 months, disease-free survival was 28% in patients with AML, 33% in those with MDS and 33% in those with ALL [42]. In comparison with conditioning without radioimmunoconjugates, the toxicity of the combined conditioning scheme was not significantly increased.

In a comparative study of 12 patients (5 AML, 5 CML, 2 ALL), the biodistribution and biokinetics were investigated after intravenous injection of 906±209 MBq $^{99m}$Tc-labelled anti-CD66 mAb BW250/183 and 760±331 MBq $^{99m}$Tc-labelled anti-CD45 mAb YTH24.5. The ratios of specific organ doses of $^{99m}$Tc-BW250/183/$^{99m}$Tc-YTH24.5 were 4 for the marrow, 1 for the kidney and 0.4 for the liver (Fig. 1) [43]. YTH24.5 accumulated in the liver to a higher degree than in the bone marrow and was therefore not appropriate for myeloablative conditioning. This may be due to nonspecific binding by Fc-receptors of hepatic macrophages or to specific binding on CD45-positive leucocytes in the liver (for example, Kupffer cells). The isotype of YTH24.5 was altered from IgG2b to IgG2a and was named anti-CD45 mAb YAML568. After pretherapeutic infusion of 0.5 mg/kg body weight of unlabelled cold YAML568, the ratio of marrow dose/liver dose improved significantly, and the antibody was suitable for radioimmunotherapy [44] (Table 4). Although the biodistribution and dosimetric data for the anti-CD45 mAb YAML568 are still less favourable than the data for anti-CD66-mAb BW250/183, this antibody represents an alternative for leukaemia patients with a high medullary tumour load or with extramedullary disease and for patients with MM.

### Table 4 Specific organ doses, marrow/organ ratios and the most common indications of different radioimmunoconjugates.

<table>
<thead>
<tr>
<th>Radioimmunoconjugate</th>
<th>Reference</th>
<th>Specific doses (Gy/GBq)</th>
<th>Dose ratios, marrow/organ</th>
<th>Most common indications</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Marrow</td>
<td>Liver</td>
<td>Kidney</td>
</tr>
<tr>
<td>$^{188}$Re-anti-CD66 mAb BW250/183</td>
<td>28</td>
<td>1.4±0.5</td>
<td>0.6±0.3</td>
<td>0.7±0.4</td>
</tr>
<tr>
<td>$^{90}$Y-MX-DTPA-anti-CD66 mAb BW250/183</td>
<td>49</td>
<td>6.8$^a$</td>
<td>3.4$^a$</td>
<td>1.3$^a$</td>
</tr>
<tr>
<td>$^{90}$Y-2B3M-DTPA-anti-CD66 mAb BW250/183</td>
<td>50</td>
<td>10.2±1.8</td>
<td>2.7±2</td>
<td>&lt;1.0$^a$</td>
</tr>
<tr>
<td>$^{90}$Y-MX-DTPA-anti-CD45 YAML568$^b$</td>
<td>44</td>
<td>6.4±1.2</td>
<td>3.9±1.4</td>
<td>1.1±0.4</td>
</tr>
</tbody>
</table>

$^a$ Standard deviations are not available from the literature.
$^b$ Anti-CD45-antibody YAML568 after pretherapeutic infusion of 0.5 mg unlabelled mAb per kilogram body weight.

planted. The mean applied bone marrow dose was 14.9 Gy. Acute toxicities were moderate. Mild nausea was the most frequent side effect. The kidneys were the dose-limiting organs (Table 4). Disease-free survival within a follow-up period of 18 months was 45% [33]. A medullary tumour cell load of <15% tends to result in an improved disease-free survival [47].

In several patients with >50–60% infiltration of the bone marrow by leukaemic blasts, the specific marrow doses were lower than the specific doses of the liver (Buchmann, unpublished data). This was most probably due to the low number of binding sites on normal haematopoietic cells. In these patients, radioimmunotherapy with $^{188}$Re-BW250/183 was not performed.

In a pilot-study of 17 patients, serum levels of TNFα did not change during myeloablative radioimmunotherapy with $^{188}$Re-BW250/183. The TNFα serum levels were in the physiological range before radioimmunotherapy, and remained normal 24 h after radioimmunotherapy with values of 7.0±4.4 pg/ml. They had increased to pathological values of 42.9±24.7 pg/ml 24 h after subsequent total body irradiation and 10.9±9.4 pg/ml 24 h after high-dose chemotherapy (Fig. 2). Thus, it was assumed that, in contrast to conventional conditioning therapies, radioimmunotherapy should not relevantly increase the incidence of acute GvHD [53].

The first radioimmunotherapeutic trial for the treatment of children with leukaemia was also performed with $^{188}$Re-BW250/183 (Fig. 3). Seven patients, five with AML and two with ALL in the age range 1 to 16 years with an expected 1-year mortality of 100% without stem cell transplantation and about 10% after conventional transplantation were treated with 8.2±4.1 GBq $^{188}$Re-BW250/183. The applied organ doses were 13.7±8.3 Gy (bone marrow), 6.6±4.4 Gy (liver), 7.3±2.3 Gy (kidney), 22±15 Gy (spleen), 0.5±0.5 Gy (lungs) and 1.4±0.7 Gy (total body). In this setting, the kidney doses were higher than the liver doses, and the kidneys were the dose-limiting organs. The additive conditioning included high-dose chemotherapy and, optionally, total body irradiation.
before allogeneic transplantation. Radioimmunotherapy applied doses in the range 5.5–30 Gy to the bone marrow. After a follow-up period of 5.1±2.9 months, six patients died within 7–331 days of transplantation. At the time of this report, one patient was still in complete clinical remission. One patient developed a haemolytic-uraemic syndrome [54].

In a phase I/II study for patients aged 55–65 years, radioimmunotherapy with the anti-CD66 mAb BW250/183 was employed as part of a reduced dose-intensity conditioning regimen prior to allogeneic stem cell transplantation. A total of 20 patients with a median age of 63 years (17 with AML, 3 with MDS) received the mAb radiolabelled with either 188Re (8 patients) or 90Y (12 patients) during conditioning. Radioimmunotherapy was feasible and safe in this elderly patient group and provided a high marrow dose of 21.9±8.4 Gy, with a significantly higher dose when 90Y was used. Therapy-related toxicity was low. The cumulative incidence of nonrelapse mortality was 25%, and the cumulative incidence of relapse was 55% at 2 years after transplantation [49]. The high rate of relapse may be associated with T-cell depleted grafts. Comparing the data from 188Re- and 90Y-labelled BW250/183, the dose distribution of 90Y-labelled anti-CD66 mAb BW250/183 was more favourable, especially with regard to the kidney doses (Table 4). The biodistribution of 90Y-BW250/183 was significantly more advantageous than that of 188Re-BW250/183 with ratios of specific marrow dose/kidney doses of 5.4 for 90Y-BW250/183 and of 2.3 for 188Re-BW250/183 [49]. Evidence of nephropathy was seen in 6 of 93 patients (6.4%) after intensified conditioning with 188Re-BW250/183 but in none of 21 patients after treatment with 90Y-BW250/183 [36]. In these approaches, 90Y was coupled to the antibody via the chelator MX-DTPA.

Improvement has also recently been reported with the use of DTPA derivatives such as the chelator 2B3M-DTPA. A first study [50] in AML patients 90Y-labelled 2B3M-DTPA anti-CD66 antibody BW250/183 demonstrated superior estimated specific organ doses than 90Y-BW250/183 with the chelator MX-DTPA (Table 4). Figure 4 shows images with 111In-anti-CD66 antibody BW250/183 (with the chelator 2B3M-DTPA) 4–72 h after injection.

Conditioning of patients with multiple myeloma

Orchard et al. reported 18 patients with MM treated in a dose-escalated phase-I trial with 90Y-labelled 2B3M-DTPA
anti-CD66 mAb BW250/183 at doses of 5 to 37.5 MBq per kilogram body weight. Of these 18 patients, 16 subsequently received high-dose melphalan and autologous transplants and 2 received fludarabine, melphalan and allogeneic transplants. At the so far highest dose level of 37.5 MBq per kilogram lean body weight, in 18 myeloma patients and 2 AML patients a dose of 25 Gy was obtained in the bone marrow, 7.4 Gy in the liver and 5.1 Gy in the spleen [50] (Table 4). Kidney and lung doses were negligible. Toxicity profiles were acceptable and phase II trials are underway.

Monotherapies or combination therapies?

In general, if different therapies are combined, the toxicities are spread to different organ systems, and side effects may be more tolerable. This results in an enhanced antitumour effect that may even overcome therapy resistance while toxicities remain tolerable [19]. Especially for highly aggressive therapies such as the myeloablative conditioning of patients before stem cell transplantation, combination therapies are standard today [19]. Details of the antibodies and nuclides used in monotherapies and combination therapies and their indications are presented in Tables 5 and 6, respectively.

Fig. 4 Planar whole-body scintigraphy images in a patient with AML in the anterior and posterior views 48 h after injection of 185 MBq $^{111}$In-anti-CD66 mAb BW250/182. Very intense accumulation is seen in the bone marrow, and minor uptake in the liver and kidneys. The scans were kindly provided from Dr. Andreas Helisch, Nuclear Medicine, and Dr. Ralf G. Meyer, Internal Medicine/Hematology, University of Mainz, Germany

For radioimmunotherapy, three combination concepts (as illustrated in Fig. 5) are discussed:

1. Combination of different antibodies

An optimal antibody specificity assures that the radioactivity is delivered to the target tissue but spares normal organs. The targeting can be efficiently modulated if radiolabelled antibodies with different binding specificities are combined (Fig. 5a). We suggest that the combination of antibodies with specificity to haematopoietic cells and with specificity to leukaemic blasts ensures intense accumulation in the bone marrow independently of the grade of leukaemic blast infiltration, and also in extramedullary tumour cells. Because the accumulation in normal organs differs between the antibodies, the nonspecific radiation exposure of organs may be decreased with combination protocols.

2. Combination of different radionuclides

The cross-fire effect determines if the energy is transferred in a small or larger tissue radius (Fig. 5b). The combination of nuclides with different cross-fire effects may ensure a high and homogeneous energy transfer in the central and also marginal target tissue. It also enables the treatment of large tumour bulks and also minimal residual disease.

3. Combination of different radioimmunoconjugates or different treatment modalities

The combination of different antibodies and different nuclides may potentiate the effects of point 1 and 2, and
adding radioimmunoconjugates into conventional conditioning schemes may increase the anticancer effects with a tolerable increase in side effects [47] (Fig. 5c).

Suggestions for protocol specifications

In general, labelling of antibodies with $^{90}$Y and $^{177}$Lu via backbone-substituted DTPA derivatives such as 2B3M is expected to be superior (Table 4). In younger patients in good general condition, they should be applied additively to dose-escalated conditioning. In elderly and comorbid patients, myeloablative radioimmunotherapy should be added to intensity-reduced conditioning therapies.

Selection of the nuclide and antibody can be optimized by individual consideration of the type of disease, the infiltration grade of the bone marrow with leukaemic blasts and the presence or absence of extramedullary tumour cells. For this reason, combining different radioimmunoconjugates may improve the therapeutic efficacy in comparison with monotherapies.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Antibodies</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monotherapies</td>
<td>Anti-CD33</td>
<td>AML, MDS: with high tumour load (infiltration of bone marrow 50–60%); with extramedullary tumour cells</td>
</tr>
<tr>
<td></td>
<td>Anti-CD45</td>
<td>AML, MDS: with high tumour load (infiltration grade of bone marrow 50–60%); with extramedullary manifestations</td>
</tr>
<tr>
<td></td>
<td>Anti-CD66</td>
<td>AML, MDS: in complete or partial remission (infiltration of bone marrow less than 25%); without extramedullary manifestations</td>
</tr>
<tr>
<td>Combination therapies</td>
<td>Anti-CD66 + anti-CD45</td>
<td>AML, MDS: with intermediate tumour load (infiltration of bone marrow 25–60%); with or without extramedullary manifestations</td>
</tr>
<tr>
<td></td>
<td>Anti-CD66 + anti-CD33</td>
<td>AML, MDS: with intermediate tumour load (infiltration of bone marrow 25–60%); with or without extramedullary manifestations</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Nuclide</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monotherapies</td>
<td>$^{90}$Y</td>
<td>Myeloablation with high-energy transfer in the centre of the bone marrow</td>
</tr>
<tr>
<td></td>
<td>$^{177}$Lu</td>
<td>Myeloablation with high-energy transfer in the margin (and also centre) of the bone marrow</td>
</tr>
<tr>
<td></td>
<td>$^{213}$Bi</td>
<td>Targeting of minimal residual disease in extramedullary locations</td>
</tr>
<tr>
<td>Combination therapies</td>
<td>$^{90}$Y and $^{177}$Lu</td>
<td>AML and MDS: homogeneous myeloablation in the centre and margin of the bone marrow; with or without minimal residual disease in extramedullary locations</td>
</tr>
<tr>
<td></td>
<td>$^{90}$Y and $^{213}$Bi</td>
<td>MM: myeloablation; targeting of malignant plasma cells in the bone marrow; targeting of circulating myeloma progenitor cells</td>
</tr>
</tbody>
</table>
reasonable in patient with a bone marrow infiltration grade higher than 25%.

Based on theoretical considerations and recent results with peptides, a combination of $^{90}\text{Y}$ and $^{177}\text{Lu}$ is advised. $^{90}\text{Y}$ may increase the dose in the centre of the bone marrow and $^{177}\text{Lu}$ in minimal residual disease in extramedullary locations (for example, hepatic infiltration or in leukaemic blasts), and also in the margin of the bone marrow. This assumption has, however, to be proven in future studies.

Patients with MM and a diffuse pattern of bone marrow infiltration Myeloablative radioimmunotherapy for the conditioning of patients with MM still remains difficult for two major reasons. First, it is postulated that there is a progenitor myeloma stem cell that is CD45-positive and circulates as a single cell in the peripheral blood. Thus, we consider that patients with MM should be treated with anti-CD45 mAb. For radiolabelling, a combination of $\alpha$- and $\beta$-emitters might be advantageous. The $\alpha$-particle emitter should ensure sufficient damage in the single progenitor cell that circulates in the blood and also in myeloma cells and myeloma progenitor cells in the margin of the marrow. The $\beta$-particle emitter should potentiate the effect in the centre of the bone marrow.

Second, malignant plasma cells may build clusters in the bone marrow with volumes of several millilitres. Sclerosis and hypoperfusion of these clusters render targeting difficult. In order to avoid insufficient doses in large plasma cell clusters, it may be that only patients with a diffuse pattern of bone marrow infiltration and clusters $<1$ ml should be included in radioimmunotherapy protocols.

Conclusion and perspectives

The published data suggest that myeloablative radioimmunotherapy has the potential to close the gap between benefit and toxicity of conditioning therapies. Myeloablative radioimmunotherapies are increasingly used to condition patients with AML, MDS and MM who are scheduled for allogeneic and autologous stem cell transplantation. They are applied additionally to conventional conditioning schemes and transfer high-energy doses into the bone marrow. They induce hypoplasia/aplasia in the bone marrow, generate space for the engraftment of reinfused stem cells and may decrease the probability of relapse. They spare organs that do not express the target antigen. Thus, myeloablative radioimmunotherapy is safe and well tolerated and should not significantly increase the rate of therapy-related mortality.

Prior to all myeloablative radioimmunotherapy, individual dosimetry has to be performed either to calculate the
therapy doses or, in dose-escalating protocols, to ensure that the dose-limiting organ doses are not exceeded. Also, definition of the maximum organ doses that must not be exceeded is advised, and dose-escalation trials will increase patient safety.

$^{90}$Y-radioimmunoconjugates are less nephrotoxic than $^{188}$Re-labelled antibodies. For monotherapy in patients with AML or MDS and a low tumour load, the $^{90}$Y-anti-CD66 mAb BW250/183 is optimal. The use of backbone-stabilized DTPA-derived chelators is suggested. They improve the biodistribution, resulting in an increase in the ratio of bone marrow doses to liver doses. In patients with a high fraction of leukaemic blasts in the bone marrow or significant extramedullary infiltration, anti-CD33 and anti-CD45 radioimmunoconjugates are preferred or applied additionally.

In patients older than 55 years as well as patients with severe pretreatment of concomitant diseases, conventional conditioning might be highly toxic and this has limited the use of stem cell transplantation so far. Myeloablative radioimmunotherapy is, however, well tolerated and can be combined with intensity-reduced conditioning without significantly increasing toxicity. Thus, it opens a new field of therapeutic approaches in elderly patients.

Acute GvHD relevantly contributes to therapy-related mortality after allotransplantation. Among other factors, it is mediated by an increase in TNF$\alpha$ levels during aggressive conditioning schemes. In contrast to total body irradiation and high-dose chemotherapy, myeloablative radioimmunotherapy with $^{188}$Re-BW250/183 does not significantly increase TNF$\alpha$ levels. In theory, it should therefore not significantly increase the incidence of conditioning-associated acute GvHD. However, this still remains to be demonstrated in prospective trials.

Although clinical data are not available so far, anti-CD45 mAb conjugates may be ideal in MM. They may also target the circulating myeloma progenitor cell, and a combination of $\alpha$- and $\beta$-emitters seems to be advantageous.

In future, myeloablative radioimmunotherapy will be easily applicable with the approval of the pharmaceutical industry. The pharmaceutical industry has already obtained a license for DTPA anti-CD66 mAb, and has initiated trials for the approval. The efficiency of conditioning schemes with and without myeloablative radioimmunotherapy has to be investigated in randomized studies. Subsequently, a potential increase in the efficiency by the combination of different radioimmunoconjugates has to be evaluated.

Acknowledgments We thank colleagues of the Department of Nuclear Medicine and the Department of Hematology of the University Hospitals of Heidelberg, Mainz and Ulm, Germany, who have supported the establishment of myeloablative radioimmunotherapy and have contributed to the care of our patients.

References

34. Loevinger R, Berman MA. A revised schema for calculating the absorbed dose from biologically distributed radionuclides. Society of Nuclear Medicine, New York; 1976, MIRD Pamphlet No. 1.
49. Ringhofer M, Blumstein N, Neuhauser B, et al. 188Re or 90Y-labeled anti-CD66 antibody as part of a dose-reduced conditioning regimen for patients with acute leukaemia or myelodysplastic