The clinical use of antibodies in haematological malignancies

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Introduction

Chemotherapy and radiotherapy have, for decades, been the conventional treatment for patients with haematological malignancies, including lymphomas [1] and leukaemias [2]. Stem-cell transplantation (particularly allogeneic) also opened new therapeutic possibilities in selected cases. More recently, the treatment of these patients with monoclonal antibodies (MoAbs) has provided an effective alternative approach, which can be combined with the above mentioned therapies [3–5].

MoAbs are the first targeted treatment against the cancer that is effective and gives the possibility of reducing non-specific toxicity. In recent years a number of new MoAbs against different targets in haematological and non-haematological malignancies, including lymphoid and myeloid tumours, have been progressively incorporated to the therapeutic armamentarium [6–11].

A review of the present status of MoAb therapy in all the haematological malignancies is very complicated, since the number of different MoAbs and, therefore, the number of clinical trials with these alone, and in combination, is exponentially increasing. Therefore, the objective of this manuscript is to review the state-of-the-art MoAb treatment, mainly focusing on those antibodies that are currently in use in clinical practice and, particularly, those already currently essential to the treatment of patients with leukaemias or lymphomas.

Targets and antibody effector mechanisms

Different antigens can be the target of MoAbs in leukaemias and lymphomas. In Table 1, some of the antigens potentially interesting in clinical practice are listed. The list is not complete because the number of possibilities is large. The optimal target for MoAb therapy would be a specific antigen present at high density on tumour cells, absent or present at low concentrations on normal cells (or present in non-critical host cells), with stable expression and with no modulation or internalization. CD20 antigen, present in most B-cell neoplasias, has all of these requisites and is the paradigm of the target molecules [6, 12, 13]. Nevertheless, even in those cases with a lack of high tumour specificity, MoAbs offer the possibility of lower toxicity, compared with conventional chemotherapies, by increasing the therapeutic index and with no overlapping toxicity with the standard drugs [6].

Native unconjugated antibodies were the first ones used in clinical practice. The mechanisms of action to kill the tumour cells include complement-dependent cytotoxicity (CDC) (affected by antibody isotype and species, and antigen density), antibody-dependent cellular cytotoxicity (ADCC) [which requires specific isotypes and adequate ratios of effector cells, including natural killer (NK) cells, macrophages and polymorphonuclear cells, to target cells] and receptor-based signalling [12–17]. In addition, certain characteristics of the antibodies may modulate their activity. Thus, a humanized (at least chimeric) MoAb will have less immune response from the host by not producing inactivating antibodies. More recently, in order to increase the anti-tumour effect, MoAbs have been conjugated either with radiation emitters or with cytotoxins [18–21]. The latter could substantially increase the cytotoxic capability, but also the toxicity for the patient.

Radio-immunotherapy with beta-emitting isotopes, such as 131I or 90Y, has the advantage of making a ‘crossfire effect’ eliminating tumour cells to which the MoAb is not directly bound, although of course, the dose-limiting toxicity to normal cells is important [10, 21]. Cytotoxins conjugated to MoAb, such as calicheamicin (anti-tumour antibiotic), or toxins such as ribosomal inhibitory proteins (e.g. ricin, gelonin) are drugs that require entry to the cell to work, but once inside are extremely toxic for tumour cells.

Another aspect to point out regarding the treatment with MoAb is the possibility of additive or synergistic effect with some of the standard chemotherapies [22, 23]. This is important since the synergistic effect could be obtained with no substantial increase in toxicity. The advances during the last years in immunotherapy of haematological malignancies derive from the combination of MoAbs with conventional chemotherapy.

Lymphoproliferative disorders

Rituximab

Rituximab is a chimeric monoclonal immunoglobulin G1 antibody of humanized murine origin and targets the cell surface receptor CD20. It was the first antibody widely used in patients with malignant and non-malignant diseases. In the lymphoma setting, since its first use in humans a decade ago, rituximab has become an essential component of the therapy in all types of B-cell lymphomas, probably representing the major advance in this field since the use of doxorubicin in the 1970s [1, 3, 4]. Alone or in combination with
The combination of rituximab plus standard chemotherapy dramatically increases the CR rate, failure-free and disease-free survival both in previously treated and untreated patients. The latter was observed with different regimens (CVP (cyclophosphamide, vincristine and prednisone) [33], CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) [34], FCM (fludarabine, cyclophosphamide and mitoxantrone)
Table 2. Pharmacological characteristics of the most widely used monoclonal antibodies in haematological malignancies

<table>
<thead>
<tr>
<th>Rituximab (MabThera®)</th>
<th>[131I]tositumomab (Bexxar®)</th>
<th>[90Y]ibritumomab tiuxetan (Zevalin®)</th>
<th>Alemtuzumab (MacCampath®)</th>
<th>Gemtuzumab ozogamicin (Mylotarg®)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target antigen</strong></td>
<td>CD20</td>
<td>CD20</td>
<td>CD20</td>
<td>CD33</td>
</tr>
<tr>
<td><strong>Type of antibody</strong></td>
<td>Chimeric</td>
<td>Radiolabeled murine</td>
<td>Radiolabeled murine</td>
<td>Recombinant humanized conjugated to calicheamicin</td>
</tr>
<tr>
<td><strong>Target disease</strong></td>
<td>B-cell NHL; CLL</td>
<td>B-cell NHL; CLL</td>
<td>B-cell NHL; CLL</td>
<td>AML</td>
</tr>
<tr>
<td><strong>Usual regimen</strong></td>
<td>Monotherapy: 375 mg/m² weekly x 4 weeks</td>
<td>Platelet count 2150 × 10⁹/l; 0.4 mCi/Kg each cycle</td>
<td>Needs dosimetry to establish the dose</td>
<td>Monotherapy: 9 mg/m² x two doses</td>
</tr>
<tr>
<td></td>
<td>Combined with CT: 375 mg/m² on 1st day of each cycle</td>
<td>Platelet count 100–150 × 10⁹/l; 0.3 mCi/Kg (Up to 32 mCi)</td>
<td>CLL; T-cell NHL 30 mg 3 times per week x8–12 weeks (starting with escalated doses: 3, 10 and 30 mg)</td>
<td>Combined with CT: 3 mg/m² day 1</td>
</tr>
<tr>
<td><strong>Main adverse effects</strong></td>
<td>Infusion toxicity (cytokine release syndrome)</td>
<td>Myelosuppression</td>
<td>Infusion toxicity</td>
<td>Myelosuppression</td>
</tr>
<tr>
<td></td>
<td>Leukaemic expression</td>
<td>Bone marrow (+)</td>
<td>Opportunistic infections</td>
<td>Opportunistic infections</td>
</tr>
<tr>
<td><strong>Cautions</strong></td>
<td>Bone marrow (+)</td>
<td>Opportunistic infections</td>
<td>Caution with thioguanine when used in combination</td>
<td></td>
</tr>
</tbody>
</table>

NHL, non-Hodgkin’s lymphoma; CLL, chronic lymphocytic leukaemia; AML, acute myeloid leukaemia; CT, chemotherapy.

This survey was confirmed in a similar group of patients in an American study [42]. Although the design was different (patients in response were randomized to no further treatment or maintenance with rituximab), those patients receiving rituximab either with CHOP or as maintenance had better survival. Moreover, the superiority of R-CHOP was shown in the setting of younger patients with low-risk DLBCL [41]. More recently, the addition of rituximab to a high-density regimen (CHOP-14) has demonstrated an improvement of response, disease-free and overall survival [44].

There are several ongoing studies on the role of rituximab in combination with other chemotherapy regimens, with autologous stem-cell transplantation, as well as the combination of different MoAbs plus rituximab-containing chemotherapy.

*chronic lymphocytic leukaemia (CLL)*. In patients with CLL, rituximab alone has a discreet effect due, in part, to the low density of CD20 antigen in the surface of CLL tumour cells [13]. Higher doses (500–2250 mg/m²) are necessary to increase the responses [51]. The combination with chemotherapy notably improves the effect of the latter. The combination of rituximab with fludarabine and cyclophosphamide shows a CR rate of about 70%, with a high proportion of patients reaching a molecular CR [52–54]. Certainly, the toxicity of the combination, especially in terms of immunosuppression, should be taken into account. The use of rituximab in maintenance therapy is currently under investigation.
alkylating agents or fludarabine-containing regimens. Cases seen were most likely related to previous therapy with myelodysplasia or acute leukaemia has been reported and the is of note that no strong evidence of higher risk of haemoglobin and platelets occurs at 5–8 weeks after the dose. It < the lymphoma. For this reason, patients with platelet count myelosuppression, especially when bone marrow is involved by unlabeled anti-CD20 (rituximab). Dosimetry estimation, mandatory for other radioisotopes, seems not to be necessary for \[^{90}Y\]ibritumomab tiuxetan \[^{10}\]. The main toxicity is limit toxicity of \[^{131}I\]tositumomab \[^{10}\]. Myelosuppression is the main con- straint for this drug. Myelosuppression is the main limitation toxicity of \[^{131}I\]tositumomab \[^{10}\].

Table 3. Overall survival in selected randomized trials in first-line treatment comparing chemotherapy (CT) alone versus CT plus rituximab (R) in patients with different types of lymphoma

<table>
<thead>
<tr>
<th>Disease</th>
<th>Reference</th>
<th>Regimen</th>
<th>Overall survival (CT (%))</th>
<th>Overall survival (CT + R) (%</th>
<th>Time of overall survival assessment</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLBCL</td>
<td>Coiffier 2002 [39] Feugier 2006 [40]</td>
<td>CHOP</td>
<td>399 45</td>
<td>58</td>
<td>5 years</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td>Habermann 2006 [42]</td>
<td>CHOP</td>
<td>632 58</td>
<td>67</td>
<td>3 years</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Pfreundschuh 2006 [41]</td>
<td>CHOP</td>
<td>824 84</td>
<td>93</td>
<td>3 years</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Pfreundschuh 2006 [54]</td>
<td>CHOP-14</td>
<td>1222 67</td>
<td>75</td>
<td>3 years</td>
<td>0.003</td>
</tr>
<tr>
<td>MCL</td>
<td>Lenz 2005 [55]</td>
<td>CHOP</td>
<td>128 76</td>
<td>76</td>
<td>2 years</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Herold 2004 [35]</td>
<td>MCP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FL</td>
<td>Marcus 2005 [33] Marcus 2006 [56]</td>
<td>CVP</td>
<td>322 77</td>
<td>83</td>
<td>4 years</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Haldeman 2005 [34]</td>
<td>CHOP</td>
<td>557 90</td>
<td>95</td>
<td>2 years</td>
<td>0.016</td>
</tr>
<tr>
<td></td>
<td>Herold 2007 [57]</td>
<td>MCP</td>
<td>201 74</td>
<td>87</td>
<td>4 years</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Ruske 2006 [58]</td>
<td>CHOP</td>
<td>221 81</td>
<td>90</td>
<td>4 years</td>
<td>0.04</td>
</tr>
</tbody>
</table>

DLBCL, diffuse large B-cell lymphoma; MCL, mantle-cell lymphoma; FL, follicular lymphoma.

\[^{90}Y\]ibritumomab tiuxetan

\[^{90}Y\]ibritumomab tiuxetan (Zevalin\textsuperscript{®}) is a conjugate of murine anti-CD20 MoAb and the radionuclide \[^{90}Y\] that delivers \(\beta\)-radiation in a 1–5 mm radium sphere. The infusion of the radiolabeled MoAb is preceded by two doses of a ‘cold’ unlabeled anti-CD20 (rituximab). Nadir of neutrophils, haemoglobin and platelets occurs at 5–8 weeks after the dose. It is of note that no strong evidence of higher risk of myelodysplasia or acute leukaemia has been reported and the cases seen were most likely related to previous therapy with alkylating agents or fludarabine-containing regimens.

\[^{90}Y\]ibritumomab tiuxetan has shown activity in relapsed FL patients, in whom CR rate and failure-free survival after this drug is longer than after rituximab [62, 63]. Noteworthy is that a proportion of relapsed patients had prolonged CR periods. At the present time, the established indication of this molecule is for relapsed or resistant to rituximab tiuxetan. In DLBCL and MCL \[^{90}Y\]ibritumomab tiuxetan shows some activity. The possible role for \[^{90}Y\]ibritumomab tiuxetan in consolidation of first response after conventional chemotherapy is currently being studied. In addition, the combination of \[^{90}Y\]ibritumomab tiuxetan with chemotherapy, including in the setting of autologous stem-cell transplantation, is also under investigation.

\[^{131}I\]tositumomab

\[^{131}I\]Tositumomab (Bexxar\textsuperscript{®}) combines a murine anti-CD20 MoAb with \(^{131}I\). The dose depends on the dosimetry that is mandatory for this drug. Myelosuppression is the main limitation toxicity of \[^{131}I\]tositumomab \(^{10}\).

In groups of patients with indolent lymphoma considered refractory to rituximab or with transformed lymphoma, \[^{131}I\]tositumomab induced overall responses rates of 65%, including 20% CR, with median response duration of 6–7 months. In untreated patients, single agent \[^{131}I\]tositumomab reaches a CR rate of 75%, with a median of progression-free survival of 6.1 years. On the other hand, \[^{131}I\]tositumomab has been combined with CHOP as consolidation, with 90% of overall response and 67% CR [64–68]. Currently there is an ongoing study that compares rituximab and \[^{131}I\]tositumomab combined with CHOP in FL patients not previously treated.

Alectuzumab

Alectuzumab is a humanized IgG1 monoclonal antibody with specificity for the CD52 antigen, a glycosylphosphatidylinositol-anchored (lymphocyte-surface glycoprotein) which is widely expressed at high density on all human lymphoid cells (except plasma cells), as well as in eosinophils, monocytes, dendritic cells and macrophages. Alectuzumab is classically administered in a 2-hour intravenous infusion. Standard dose is 3 mg (1st day), 10 mg (2nd day) and 30 mg (3rd day); if it is well tolerated, 30 mg will be administered 3 days per week for 8–12 weeks. Subcutaneous administration is being increasingly used because the infusion syndrome is less frequent and does not need escalation of the dose. Pre-medication is usually given to prevent side effects of infusion (headache, mouth sores, rash, low blood pressure and fatigue), including dexchlorpheniramine, acetaminophen and hydrocortisone.

Immunosuppression is the most important toxic effect, but it is manageable. Prophylaxis against Pneumocystis carinii should be used and prophylaxis against herpes zoster/simplex viral infections should also be considered. Weekly monitoring with cytomegalovirus polymerase chain reaction testing is recommended \[^{10}\].

At present, alectuzumab is licensed for patients with CLL, previously treated with alkylating agents and refractory to fludarabine. In this setting, reports shows that up to 87% of previously untreated patients respond to alectuzumab with a 17% CR. Combination with other drugs, including fludarabine and rituximab, is the subject of in-progress investigations [69–72]. Strong immunosuppression is the main concern. Some responses have also been attained in refractory T-cell prolymphocytic leukaemia. Moreover, peripheral T-cell lymphomas constitute a group of poor-risk
patients in whom alemtuzumab alone or in combination may have an important role [69, 70]. Lastly, in the setting of allogeneic transplantation, alemtuzumab is used in order to do the T-cell depletion.

denileukin diftitox
Denileukin diftitox is a fusion protein that targets the diphtheria toxin to cells expressing the interleukin-2 receptor (CD25). When internalized into the cell, the drug inhibits protein synthesis. This MoAb has been used in cutaneous T-cell lymphoma, refractory B and T-cell lymphomas and in fludarabine-resistant CLL [73].

other antibodies
Due to the content limitation required for this review it would be impossible to mention many other MoAbs that are being used in clinical trials. However, among these it would be of interest to mention the following molecules: the new different anti-CD20 MoAb (including the fully humanized IMMU-106 hA20), anti-CD22 (unconjugated epratuzumab and calicheamicin conjugated CMC-544), anti-CD30 (SGN-30 and iratumumab), anti-CD40 (SGN-40), anti-CD80 (galiximab), anti-CD2 (sipiluzumab) and anti-CD4 (L3T4). Bevacizumab, a VEGF (vascular endothelial growth factor) inhibitor may play also an interesting anti-lymphoma role in the future.

acute leukaemias
Table 1 shows a list of potential targets to block by MoAb in acute leukaemias. Gemtuzumab ozogamicin is nowadays the only one being used in the clinical practice out of clinical trials.

gemtuzumab ozogamicin
Gemtuzumab ozogamicin is an immunoconjugate of an anti-CD33 antibody chemically linked to a potent cytotoxic agent, calicheamicin. It appears to be particularly active in patients with acute leukaemia. CD33 antigen is expressed on the surface of leukaemic cell blasts in more than 90% of patients with acute myeloblastic leukaemia [7]. It is administered as a 2-hour intravenous infusion. The dose infused is 6–9 mg/m² in two administrations. Side effects include myelosuppression, headache, rash, low blood pressure, increased levels of hepatic enzymes and fatigue. A unique potential toxicity is a veno-occlusive-like disease that may be very severe and always problematic in patients who subsequently undergo a haematopoietic stem cell transplantation.

As single agent treatment (9 mg/m² in two doses), 26% of patients receiving gemtuzumab ozogamicin reached CR that lasted for a median of 6 months [74]. The feasibility of combining gemtuzumab ozogamicin (at a dose of 3 mg/m²) with chemotherapy has been recently demonstrated with a reasonable toxicity [75]. In this sense, regimens containing thioguanine should be avoided because of grades 3–4 liver toxicity.

conclusion
Much progress has been made during the last few decades in the treatment of haematological malignancies. MoAbs represent a major advance towards a targeted therapy that can dramatically improve the anti-tumour effect with a substantial reduction of toxicity derived from therapy. In general, MoAbs are safe, well-tolerated, and have activity in a variety of clinical settings. The combination with chemotherapy as well as the combination of different MoAbs is not a dream for the future, but a solid reality for many patients with lymphoma or leukaemia.

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