Radioimmunotherapy Using $^{131}$I-labeled Anti-CD22 Monoclonal Antibody (LL2) in Patients with Previously Treated B-cell Lymphomas

Ola Lindén,1 Jan Tennvall, Eva Cavallin-Ståhl, Lennart Darte, Michael Garkavij, Karl-Johan Lindner, Michael Ljungberg, Tomas Ohihsson, Katarina Sjögren, Karin Wingårdh, and Sven-Erik Strand

Departments of Oncology [O. L., J. T., E. C.-S., M. G.], Radiation Physics [L. D., M. L., T. O., K. S., K. W., S.-E. S.], and Hospital Pharmacy [K.-J. L.], Lund University Hospital, SE-221 85 Lund, Sweden

Abstract

Experience in using rapidly internalizing antibodies, such as the anti-CD22 antibody, for radioimmunotherapy of B-cell lymphomas is still limited. The present study was conducted to assess the efficacy and toxicity of a $^{131}$I-labeled anti-CD22 monoclonal antibody (mAb), LL2, in patients with B-cell lymphomas failing first- or second-line chemotherapy. Eligible patients were required to have measurable disease, less than 25% B cells in unseparated bone marrow, and an uptake of $^{99m}$Tc-labeled LL2Fab' in at least one lymphoma lesion on immunoscintigram. Eight of nine patients examined with immunoscintigraphy were unequivocally found to have an uptake, and therapy with $^{131}$I-labeled anti-CD22 [1330 MBq/m² (36 mCi/m²)] preceded by 20 mg of naked anti-CD22 mAb was administered. Three patients achieved partial remission (duration, 12, 3, and 2 months), and one patient with progressive lymphoma showed stable disease for 17 months. Four patients exhibited progressive disease. The toxicity was hematological. Patients with subnormal counts of neutrophils or platelets before therapy seemed to be more at risk for hematological side effects. Radioimmunotherapy in patients with B-cell lymphomas using $^{131}$I-labeled mouse anti-CD22 can induce objective remission in patients with aggressive as well as indolent lymphomas who have failed prior chemotherapy.

Introduction

Non-Hodgkin’s lymphomas can be extremely sensitive to external radiotherapy, as evidenced by fractionated whole body radiotherapy, in which mean total absorbed doses below 2 Gy have been reported to induce a substantial number of responses (1, 2). There is increasing interest in the treatment of B-cell lymphomas with naked mAbs, which may induce long-lasting remission, especially in indolent lymphomas (3). When using RIT, i.e., radiolabeled mAbs, higher response rates have been reported in selected series including indolent as well as aggressive lymphomas (4). CR rates of 50% have been published in the nonmyeloablative RIT setting (5), and CR rates of 76% have been published with RIT with stem cell support (6).

Most investigators of the use of radiolabeled mAb and naked mAb for the treatment of B-cell lymphomas have used an anti-CD20 antibody (3–6). The vast majority of RIT studies also include a high preload of naked antibody to achieve a more favorable distribution of the subsequent radiolabeled antibody. The naked antibody might also mediate cell killing by immunological effector functions and possibly by direct induction of apoptosis (7).

The CD22 antigen has been implicated as a negative regulator of antigen receptor signaling (8). The antigen is expressed during B-cell development from the immature B cell to the activated B-cell stage (9) and on most B-cell lymphomas; thus, it is not expressed on stem cells or on plasma cells. The antigen is rapidly internalized after binding by the antibody. The antigen is then rapidly reexpressed on the cell surface. It has been estimated that approximately 50% of the internalized antigen is available for binding after 5 h (10).

Experience in RIT using a rapidly internalizing antibody, such as the anti-CD22 mAb (11, 12) is still limited. The present study was conducted to assess the efficacy and toxicity of $^{131}$I-labeled anti-CD22 mAb in a series of patients with B-cell lymphomas failing standard chemotherapy.

Materials and Methods

Radioimmunoagents. The LL2 mAb (Immunomedics, Inc., Morris Plains, NJ) is a murine IgG2a directed against the CD22 antigen. The LL2 Fab' fragment for immunoscintigraphy (LymphoScan) was obtained as a lyophilized powder that was reconstituted with approximately 1100–1300 MBq of $^{99m}$Tc. The radiochemical purity was determined before injection using TLC. The radiochemical purity should not be less than 90%. $^{131}$I-labeled LL2 mAb was used for dosimetric studies as well as for RIT.

1 Presented at the “Seventh Conference on Radioimmunodetection and Radioimmunotherapy of Cancer,” October 15–17, 1998, Princeton, NJ. Supported by grants from the Swedish Cancer Society; Mrs. Berta Kamrad Foundation; the Gunnar, Arvid, and Elisabeth Nilsson Foundation; Gustaf V Jubilee Fund; the Medical Faculty, University of Lund; and Foundations of Lund’s Health District Organization.

2 To whom requests for reprints should be addressed, at Department of Oncology, University Hospital, Lund, SE-221 85 Lund, Sweden. Phone: 46-46-17-75-20; Fax: 46-46-18-81-43; E-mail: ola.lind@onk.lu.se.

3 The abbreviations used are: mAb, monoclonal antibody; RIT, radioimmunotherapy; REAL, revised European-American lymphoma classification; HAMA, human antimouse antibody; CR, complete remission; PR, partial response; SD, stable disease; DLBC, diffuse large B cell lymphoma; DLBC-Tf, DLBC transformed; B-CLL, B-chronic lymphocytic leukemia lymphoma; HDCT, high-dose chemotherapy with stem cell rescue; CT, computed tomography.
Radioiodination with $^{131}$I was performed according to the Iodogen method (13). Briefly, the antibody was added together with a buffer solution to a vial; the inner walls of the vials were coated with iodogen. The radioiodination procedure was terminated by the addition of an anion exchange resin. Thereafter, the mixture was passed through a filter with a nominal pore size of 0.22 μm into a vial with albumin and buffer. The specific activity was approximately 370 MBq/mg antibody. High-performance liquid chromatography was used for determination of the identity of the labeled antibody as well as radiochemical purity. An additional test to determine the amount of free iodine in the radiopharmaceutical was performed using TLC. The radiochemical purity should be less than 95%.

**Patient Selection.** All patients with a diagnosis of B-cell lymphoma established on a tissue biopsy could be included. A histopathological review was performed, and the lymphoma was classified according to the REAL lymphoma classification (14). All patients should have failed one or two chemotherapy regimens, which might include a consolidation with HDCT. Further inclusion criteria were as follows: (a) measurable disease; (b) less than 25% B cells in unseparated bone marrow as analyzed by flow cytometry; (c) a maximal diameter of lymphoma lesions and spleen of 10 and 20 cm, respectively; (d) an absolute granulocyte count exceeding 1,500/μl; (e) a platelet count exceeding 100,000/μl; (f) normal hepatic and renal function; (g) Eastern Cooperative Oncology Group performance status of 0 or 1; (h) life expectancy of at least 3 months; and (i) no HAMAs. Finally, all patients were required to give written informed consent for participation in the study.

**Study Design.** If all of the criteria given above were fulfilled, the patient was eligible for inclusion in the study starting with an immunoscintigram using $^{99m}$Tc-LL2Fab’. All patients underwent a CT of the thorax, abdomen, and pelvis; and bone marrow biopsy if previously positive for lymphoma was performed 6 weeks after therapy and every 2-3 months month thereafter. CR was defined as a complete disappearance of all detectable disease for at least 1 month. PR was defined as a reduction of at least 50% in the sum of the products of the largest perpendicular diameters in all lesions for at least 1 month, without the appearance of new lesions. Patients were defined as having SD if they exhibited < 50% decrease in all lesions or <25% increase in any lesions and did not develop any new lesions.

**Toxicity Criteria.** Toxicity was scored according to the National Cancer Institute Common Toxicity Criteria. Dose-limiting hematological toxicity was defined as any grade 3 toxicity that did not return to the baseline level within 10 weeks and any grade 4 toxicity. Blood counts and blood chemistries were checked weekly (or more often, if indicated) for the first 10 weeks and checked later at regular visits.

**Results**

Between November 1996 and April 1998, nine patients were enrolled in the study and investigated with a scintigram, using $^{99m}$Tc-labeled LL2Fab’ (average radiochemical purity, 98% ± 1%). One week later, treatment with 1330 MBq/m² (36 mCi/m²) $^{131}$I-labeled antibody, preceded by 20 mg of naked LL2 antibody, was administered. Both $^{131}$I-labeled antibody infusions were followed by multiple blood sampling and gamma camera investigations for later pharmacokinetic and dosimetric studies. Premedication with potassium iodine, potassium perchlorate, and allopurinol was always given. Treated patients were isolated until their dose rate was less than 35 μSv/h at a distance of 1 m from the patient.

Patients were considered for retreatment within 6–10 weeks if they had SD or regression and a hematological toxicity of grade 3 or less that had returned to the baseline value within 10 weeks. A further criterion for retreatment was a negative HAMA titer.

**Response Criteria.** A complete reevaluation of disease status including physical examination; CT of the thorax, abdomen, and pelvis; and bone marrow biopsy if previously positive for lymphoma was performed 6 weeks after therapy and every 2-3 months month thereafter. CR was defined as a complete disappearance of all detectable disease for at least 1 month. PR was defined as a reduction of at least 50% in the sum of the products of the largest perpendicular diameters in all lesions for at least 1 month, without the appearance of new lesions. Patients were defined as having SD if they exhibited < 50% decrease in all lesions or <25% increase in any lesions and did not develop any new lesions.

**Table 1** Patient characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Gender</th>
<th>Age (yr)</th>
<th>REAL classification</th>
<th>Stage at RIT</th>
<th>Prior treatment</th>
<th>Chemosensitivity</th>
<th>Disease status at RIT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>58</td>
<td>DLBC</td>
<td>II</td>
<td>CHOP MIME HDCT</td>
<td>XRT No</td>
<td>P</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>70</td>
<td>B-CLL</td>
<td>IV</td>
<td>CNOP</td>
<td>No</td>
<td>P</td>
</tr>
<tr>
<td>3</td>
<td>Male</td>
<td>60</td>
<td>DLBC</td>
<td>III</td>
<td>CHOP MIME</td>
<td>XRT No</td>
<td>P</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>58</td>
<td>F1 grade II</td>
<td>IV</td>
<td>Chl CHOP</td>
<td>No</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>26</td>
<td>IC</td>
<td>III</td>
<td>Chl</td>
<td>No</td>
<td>No RIT</td>
</tr>
<tr>
<td>6</td>
<td>Male</td>
<td>75</td>
<td>DLBC</td>
<td>II</td>
<td>CHOP</td>
<td>Yes</td>
<td>P</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>55</td>
<td>Indolent nonclassifiable</td>
<td>II</td>
<td>Chl</td>
<td>Yes</td>
<td>P</td>
</tr>
<tr>
<td>8</td>
<td>Male</td>
<td>64</td>
<td>DLBC-Tf</td>
<td>III</td>
<td>CHOP MIME HDCT</td>
<td>XRT Yes</td>
<td>P</td>
</tr>
<tr>
<td>9</td>
<td>Female</td>
<td>75</td>
<td>DLBC</td>
<td>IV</td>
<td>CHOP EChlP</td>
<td>No</td>
<td>P</td>
</tr>
</tbody>
</table>

* Tumor histology was classified according to the REAL lymphoma classification: F1 grade II, follicular lymphoma grade II; IC, immunocytoma.

* Prior treatment: XRT, external beam radiotherapy; CHOP, cyclophosphamide-doxorubicin-vincristine-prednisone; CNOP, cyclophosphamide-mitoxantrone-vincristine-prednisone; Chl, chlorambucil; MIME, methylGaG-ifosfamide-metotrexate-etoposide; EChlP, etoposid-chlorambucil-prednisone.

* Chemosensitivity is defined as an objective response, CR or PR lasting ≥1 month after last chemotherapy.

* P, progressive; R, residual disease at RIT.
Fig. 1  Immunoscintigrams performed at 4 and 24 h after i.v. injection of $^{99m}$Tc-labeled LL2 Fab' into a responder (patient 4.). The whole body activity has considerably decreased at 24 h postinjection, when the largest lesion is clearly visible (arrow). The high uptake in the kidneys at 24 h reflects the renal reabsorption of the $^{99m}$Tc-labeled LL2 Fab'.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>RIT with $^{131I}$-anti CD22: response and duration versus histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient no.</td>
<td>REAL classification$^a$</td>
</tr>
<tr>
<td>1</td>
<td>DLBC</td>
</tr>
<tr>
<td>2</td>
<td>B-CLL</td>
</tr>
<tr>
<td>3</td>
<td>DLBC</td>
</tr>
<tr>
<td>4</td>
<td>F1 grade II</td>
</tr>
<tr>
<td>5</td>
<td>IC</td>
</tr>
<tr>
<td>6</td>
<td>DLBC</td>
</tr>
<tr>
<td>7</td>
<td>Indolent</td>
</tr>
<tr>
<td>8</td>
<td>DLBC-Tf</td>
</tr>
<tr>
<td>9</td>
<td>DLBC</td>
</tr>
</tbody>
</table>

$^a$ Tumor histology was classified according to the REAL lymphoma classification: F1 grade II, follicular lymphoma grade II; IC, immunocytoma. $^b$ PD, progressive disease; SD, stable disease; PR, partial response.

DLBC-Tf. One patient had follicular lymphoma grade II, one had B-CLL with evidence of blast transformation, and one had indolent lymphoma, which was nonclassifiable. The patient who had an immunoscintigram that was difficult to interpret had a lymphoplasmacytoid lymphoma/immunocytoma. At the start of treatment, three patients had subnormal counts for neutrophils or platelets. None of these patients and only one of the other patients (patient 2) had bone marrow infiltration. One patient (patient 4) had splenomegaly, and one (patient 8) had undergone splenectomy.

The prescribed activity of $^{131I}$ (1330 MBq/m$^2$) for the first cycle of RIT could be administered to six patients. Two patients, patients 8 and 9, received only 67% of the prescribed activity due to suboptimal radiolabeling. The activity of free iodine never exceeded 105 MBq, corresponding to a radiochemical purity of 94% (range, 94–99%).

Tumor Response

Treatment outcome related to the type of lymphoma is shown in Table 2.

Responders. Three patients achieved PR. Patient 4 had follicular lymphoma grade II, which had responded with less than a PR to a previous anthracycline-based regimen, and achieved a PR lasting 12 months. She was retreated twice with half of the initial activity; the reduction was made due to hematological toxicity. Progression of the disease took the form of the development of a lesion that was not visible at the start of treatment. Other lesions remained in remission. Patient 6 had DLBC recurring more than 5 years after his primary treatment. He responded with a PR lasting 3 months. Due to the development of HAMA, he had to be retreated with humanized LL2 outside the protocol. The patient developed a fatal condition of hemophagocytosis (15), and the autopsy revealed persisting lymphoma. Patient 8 had DLBC-Tf relapsing after HDCT. His first course of RIT, to which he responded with a PR lasting 2 months, contained only 67% of the prescribed activity. He was retreated with 50% activity due to thrombocytopenia, but he developed a new lesion, whereas the other lesions remained in remission. Two of the patients achieving a PR, patients 6 and 8, had a rapid tumor regression within less than 2 weeks, in
contrast to patient 4, who achieved a PR 4 months after the start of RIT. Patient 2 with B-CLL, who had progressive disease during primary chemotherapy, had SBD for 17 months after RIT. She developed HAMA, but she was retreated with mouse LL2 after 1 year, when the titer had declined below 200 μg/liter.

**Nonresponders.** In patients 1, 3, 7, and 9, the disease progressed during RIT. Patient 1 with DLBC relapsed less than a year after HDCT. He had also undergone external beam radiotherapy and exhibited progressive disease within the radiated volume on admission. Patient 3 with DLBC, who had not responded to prior chemotherapy, had rapidly progressing disease, also within the target volume of previous external beam radiotherapy. Patient 7 had an indolent lymphoma and responded to previous chemotherapy with a PR of short duration. The disease progressed not only after RIT but also during subsequent chemotherapy. Patient 9 with DLBC had rapidly progressing disease at RIT 5 weeks after the last course of chemotherapy. The patient received only 67% of the prescribed activity and continued to progress rapidly and started chemotherapy.

**Toxicity.**

Two patients developed HAMA (see above). Nonhematological toxicity was mild and consisted of minor reversible elevations of liver function tests. Six of eight patients were evaluable for hematological toxicity. One patient (patient 3) died of progressive disease 4 weeks after RIT, and another (patient 9) started chemotherapy before completing the first course of RIT, which precluded these patients from evaluation.

Three patients (patients 2, 6, and 7) had only transient grade 3 or grade 2 hematological toxicity. Three other patients (patients 1, 4, and 8) already had subnormal blood counts of neutrophils or platelets at the start of treatment, and two patients suffered grade 3 and one patient suffered grade 4 toxicity that did not return to the baseline value within the follow-up period. One of these three patients (patient 4) showed evidence of hemolysis before as well as after RIT, which probably contributed to the toxicity.

**Discussion.**

Investigators from several institutions have reported encouraging but varying remission rates after RIT (4–6, 16, 17). These studies differ in their choice of radionuclide, amount of administered activity, antibody, and patient selection. Although higher activity (MBq) RIT has tended to be more efficacious, no direct correlation between activity and response has been demonstrated (18). There is a clear tendency for the indolent and transformed lymphomas to be more sensitive to RIT (5). In the current study of eight patients with clinically aggressive as well as indolent B-cell lymphomas, all but one were in a progressive phase at the time of treatment, three cases of PR and one case of SBD (for 17 months) were achieved by means of 131I-labeled internalizing mouse anti-CD22. This shows that RIT with this internalizing mAb can induce objective remission in patients with different types of lymphoma after the failure of previous chemotherapy. The present results appear to be promising, considering the unfavorable patient population treated; five heavily pretreated patients had aggressive (DLBC) lymphomas, and only one of the other three patients had achieved an objective response to previous chemotherapy.

A drawback of the CD22 antigen as a target in RIT using the present 131I-labeled technique of mAb is the fast internalization (19) and consequent dehalogenation of the antibody, resulting in swift elimination of the radionuclide from the tumor target. This results in a lower absorbed dose (20), which might thwart the therapeutic benefit to the patient. This problem is circumvented by using residualizing radiolabels, such as radioactive metals (17), which are retained longer intracellularly (20). Through 90Y labeling of (humanized) LL2, the absorbed radiation dose to the tumor is reported to 4.7 times higher than that with 131I labeling of the same antibody (20).

The amount of cold antibody was chosen for an optimal targeting of 131I anti-CD22. Preliminary data indicated that 20–50 mg might be the optimal dose (11).

Most investigators use a high preload of naked mAb (up to 685 mg) to achieve a favorable distribution of the subsequent radiolabeled antibody. This dose of naked mAb may also mediate inhibition of proliferation and induction of apoptosis (7) as well as antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity. In the present study, a low amount of naked mouse LL2 antibody was administered (20 mg) before the radiolabeled one. The mouse origin of the mAb is likely to make it less effective in activating cytotoxic mechanisms (21). The low amount and the origin of the antibody used make it probable that the major effect of the antibody is confined to targeting of the radionuclide.

While remaining in remission at locations involved by tumor at the time of treatment, two of the responders (patients 4 and 8) showed progressive disease elsewhere. This pattern of progression, which was reported previously by Kaminski et al. (5), is different from the pattern of failure seen after high-dose chemotherapy (22) and might be due to a low absorbed radiation dose in the very minimal disease manifestations. It has been estimated that the highest probability of tumor eradication when using targeted 131I therapy occurs at a tumor diameter of 3.4 mm (23), indicating that most energy is deposited outside the smaller tumor cell clusters. Because the CD22 antigen is rapidly expressed on the cell surface after RIT, more frequent administration of radiolabeled mAb using lower activities (MBq) might be more effective. The study by Vose et al. (12) examining the effect of 131I-labeled mouse LL2 in 20 patients with aggressive as well as indolent B-cell lymphomas, most of whom had failed at least three chemotherapy regimens, is of great interest. These patients received 2–8 biweekly administrations of 555 MBq/m² 131I-labeled LL2 together with 20 mg of unlabeled LL2 mAb on each occasion. Responses to therapy included a CR or CR(unconfirmed) in five patients, a PR in two patients, and SBD in three patients. Several of these responses were long-lasting, indicating that this treatment schedule with mouse LL2 might be more favorable.

A humanized form of LL2 has recently been developed. The humanization process replaces approximately 95% of the murine IgG sequence with a human IgG1 sequence. The immunological effector functions might be improved by the humanization of the mAb, thus improving the therapeutic effect on single cells and tumor cell clusters (24). Based on the above-
mencioned considerations, a study using 90Y-labeled humanized LL2 administered weekly over 2–4 weeks is to be initiated.

Acknowledgments

We thank Prof. David Goldenberg (Immunomedics, Inc., Morris Plains, NJ) for kindly providing the antibody used.

References

Radioimmunotherapy Using $^{131}\text{I}$-labeled Anti-CD22 Monoclonal Antibody (LL2) in Patients with Previously Treated B-cell Lymphomas

Ola Lindén, Jan Tennvall, Eva Cavallin-Ståhl, et al.

*Clin Cancer Res* 1999;5:3287s-3291s.

Updated version
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/5/10/3287s

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.