Antiproliferative activity of a humanized anti-CD74 monoclonal antibody, hLL1, on B-cell malignancies

Rhona Stein, Zhengxing Qu, Thomas M. Cardillo, Susan Chen, Adriane Rosario, Ivan D. Horak, Hans J. Hansen, and David M. Goldenberg

The humanized anti-CD74 monoclonal antibody (mAb) hLL1 is under evaluation as a therapeutic agent. The effects of hLL1—at times in comparison with the CD20 mAb rituximab—were assessed on non-Hodgkin lymphoma (NHL) and multiple myeloma (MM) cell lines and in tumor-bearing SCID mice. In vitro, hLL1 caused growth inhibition and induction of apoptosis in B-cell lines when cross-linked with an antihuman immunoglobulin G (IgG) second antibody. The sensitivity profile of the cell lines was different for hLL1 and rituximab, and antiproliferative activity was augmented when the 2 mAbs were combined. Unlike rituximab, hLL1 did not induce antibody-dependent cellular cytotoxicity or complement-mediated cytotoxicity. In xenograft models of NHL and MM, treatment with hLL1 yielded significant survival benefits without cross-linking agents. Efficacy was greater in the MM model, in which median survival time was increased more than 4.5 fold. Thus, hLL1 has therapeutic potential as a naked mAb for B-cell malignancies because of high antigen expression on malignant cells, specifically MM, with limited expression on normal tissue, and because of its antiproliferative activity. Further, hLL1 may be a therapeutic candidate for rituximab-resistant disease because the 2 antibodies apparently act through distinct mechanisms and exhibit different expression and sensitivity profiles, and activity can be augmented when the mAbs are combined. (Blood. 2004;104:3705-3711)

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fibrous histiocytoma). CD74 expression in many of these cancers has been suggested to be a prognostic factor, with higher relative rates of CD74 behaving as a marker of tumor progression. This correlation may be related to suppressive effects on host immune responses.

A humanized form of LL1, hLL1, has been generated by complementarity-determining region (CDR) grafting, and it exhibits antigen-binding and internalization properties comparable to those of the parental murine antibody. In this paper, the naked humanized anti-CD74 mAb hLL1 is shown to cause specific in vitro growth inhibition and induction of apoptosis in B-cell lines in the presence of a second cross-linking antibody. In addition, significant survival extensions were observed in NHL- and MM-bearing SCID mice treated with naked hLL1 without the need for an exogenous cross-linking agent. In comparisons between hLL1 and the chimeric anti-CD20 mAb rituximab, we observed that the 2 antibodies act through distinct mechanisms and exhibit different expression and sensitivity profiles on B-cell malignancies. Importantly, antiproliferative activity can be augmented when the anti-CD74 and anti-CD20 mAbs are combined.

### Materials and methods

#### Cells

The Burkitt lymphoma lines Daudi, Raji, and Ramos were purchased from the American Type Culture Collection (ATCC; Manassas, VA). Non-Burkitt lymphoma cell lines were obtained as follows: RL from Dr John Gribben (Dana-Farber Cancer Institute, Boston, MA), SU-DHL-6 from Dr Alan Epstein (University of Southern California, Los Angeles), DoHH2 and WSU-FCCFL from Dr Mitchell Smith (Fox Chase Cancer Center, Philadelphia, PA), and SU-DHL-4 and Karpos422 from Dr Myron Czuczman (Roswell Park Cancer Institute, Buffalo, NY). MM cell lines were obtained as follows: ARK, ARD, and WSU-FCCL from Dr Mitchell Smith (Fox Chase Cancer Center, Philadelphia, PA), and SU-DHL-6 from Dr Alan Epstein (University of Southern California, Los Angeles). Cells were grown as suspension cultures in Dulbecco modified Eagle medium (DMEM; Life Technologies, Gaithersburg, MD) and were supplemented with 10% fetal bovine serum, penicillin (100 U/mL), streptomycin (100 

#### Antibodies

The parental murine anti-CD74 mAb LL1 (formerly called EBP-1) was generated using the Raji cell line as the immunogen source. Development of hLL1, the humanized anti-CD74 mAb, was performed by methods similar to those described previously for other humanized mAbs, including epratuzumab (hLL2), hRS7, and IMMU-106.15-18 Briefly, the genes encoding VH and VH sequences of the parent murine LL1 were cloned by reverse transcription–polymerase chain reaction (RT-PCR) and 5′-RACE, respectively, and the sequences were determined by DNA sequencing. A chimeric LL1 (cLL1) IgG containing human light- and heavy-chain constant region domains was generated and was shown to have antigen-binding specificity and affinity comparable to those of the parental LL1, confirming the authenticity of the cloned V genes. The V genes of CDR-grafted (or humanized) LL1 were then designed and engineered by a combination of long DNA oligonucleotide synthesis and PCR.15,16 hLL1 was expressed in Sp2o-Ag14 cells (ATCC) by transfection. A high-level hLL1-producing clone was developed using the procedures described previously.16 hLL1 was produced in a bioreactor and was purified by a combination of affinity chromatography on protein A columns and ion-exchange chromatography on Q-Sepharose columns.

Rituximab was purchased from IDEC Pharmaceuticals (San Diego, CA), IMMU-106 (humanized anti-CD20) and hMN-14 (humanized anti-CD66e, ant carcinoembryonic antigen IgG1), used here as a negative isotype control, were provided by Immunomedics.

#### Immunophenotyping

Indirect immunofluorescence assays were performed with the panel of cell lines described above, using murine LL1 (CD74, IgG1) and 2B8 (CD20, IgG2a; ATCC) as the primary antigen-specific mAbs and fluorescein isothiocyanate (FITC)–goat antimouse (GAM) IgG (Tago, Burlingame, CA), essentially as described previously.20 and analyzed by flow cytometry using a FACScalibur (Becton Dickinson, San Jose, CA).

#### In vitro cell proliferation assays

Effects of mAbs on cell growth were determined by assessing [3H]thymidine incorporation in the NHL and MM cell lines with and without the presence of a cross-linking second antibody, essentially as described by Shan et al.21 Second antibodies used for evaluating the effects of cross-linking were F(ab′)2 GAM IgG Fcy-specific or F(ab′)2 goat antihuman (GAH) IgG Fcy-specific (Jackson Laboratories, West Grove, PA). All tests were performed in triplicate. Statistical analyses were performed using the Student t test.

#### Analysis of apoptosis

Flow cytometric analysis of cellular DNA was performed after propidium iodide staining.21,22 Cells were placed in 24-well plates (5 × 10^5 cells/well) and were subsequently treated with mAbs (5 

#### Cytotoxicity assays

Standard chromium-51 (^51Cr) release assays were performed in triplicate to measure antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), essentially as described.23 Blood specimens used in these studies were collected under a protocol approved by the Institutional Review Board. All blood donors gave voluntary written informed consent. Normal human serum complement was purchased from Quidel (San Diego, CA). For the CMC assay, 25 

#### In vivo effects of naked mAbs on SCID mice bearing disseminated tumors

For studies on the therapeutic effect of hLL1 in NHL-bearing severe combined immunodeficient (SCID) mice (female, 6-8 weeks old; Charles River Laboratories, Frederick, MD), the animals were injected intravenously with 1 × 10^5 Raji cells or 1 × 10^7 Daudi cells. For studies in an MM model, SCID mice were pretreated with 0.4 mg/mouse Fluudara (Florida Infusion, Palm Harbor, FL) and 2 mg/mouse Neosar (Florida Infusion) 3 days before intravenous injection of 1 × 10^7 MC/AR tumor cells. After the injections of tumor cells according to the dose schedules described, mAb administration was initiated. Mice were examined daily for signs of distress or hind leg paralysis and were weighed weekly. Paralysis of the...
hind legs or a weight loss of more than 25% was used as the survival end point. Animals were humanely killed at these end points. Animal studies were performed under protocols approved by the Institutional Animal Care and Use Committee. Statistical analyses were performed using the log rank test.

Results

Construction of hLL1

The gene sequences encoding the V<sub>H</sub>/H9260 and VH of LL1 were cloned by RT-PCR and 5'-RACE, respectively. Before the process of humanization, the authenticity of the genes was confirmed by construction and expression of a chimeric LL1 IgG molecule composed of the cloned mouse V genes and human constant domain sequences. Chimeric LL1 competed with the murine LL1 for cell surface antigen binding, and the apparent avidities of these 2 antibodies were similar (data not shown). LL1 mAb was humanized by CDR grafting and transfection according to procedures similar to those used in the humanization of other murine antibodies. It should be noted that the humanized hLL1 was designed to have the same human IgG1 constant regions and hinge sequences as those of other humanized antibodies, including epratuzumab (anti-CD22), IMMU-106 (anti-CD20), and hRS7 (anti-epithelial glycoprotein-1 mAb). Antigen-binding specificity and affinity of hLL1 were confirmed to be comparable to those of the parental chimeric LL1, cLL1 (not shown).

Cell surface antigen expression

CD74 and CD20 antigen expression levels were determined by flow cytometric analysis of NHL and MM cell lines and are summarized in Table 1. The NHL cell lines include Burkitt lymphoma and several non-Burkitt lymphoma lines, most of which have the Bcl-2 gene rearrangement t(14;18). In the NHL cell lines, CD74 was expressed on the cell surface of all the cell lines tested; however, intensity varied between the cell lines, and staining was generally lower than it was for CD20 levels. In contrast, CD74 was detected on a greater number of MM cell lines than CD20, with 4 of 6 CD74<sup>+</sup> MM cell lines compared to 2 of 6 CD20<sup>+</sup> MM cell lines in the panel evaluated.

Antiproliferative effects

Antiproliferative effects of hLL1 were evaluated using [3H]-thymidine uptake assays and were compared to the activity of the anti-CD20 mAb rituximab. As shown in Figure 1A, incubation with hLL1 caused specific inhibition of proliferation in 5 of 6 NHL cell lines in the presence of a cross-linking second antibody. The sensitivity profile of the cell lines was different for hLL1 and rituximab (Figure 1B). For example, in the absence of cross-linking, no antiproliferative activity was seen with hLL1 in any cell line, whereas rituximab yielded approximately 23% inhibition of [3H]-thymidine incorporation in Daudi and Ramos cells and 88% inhibition in SU-DHL-6 cells. However, significant augmentation of the antiproliferative effects of rituximab was also observed with cross-linking. This is shown in Figure 1B, where the activity of rituximab was increased by the addition of a second antibody in 5 of 6 NHL lines and in Figure 1D in 2 of 2 CD20<sup>+</sup> cell lines from among the 6 MM cell lines tested. Interestingly, the variation in level of inhibition was not correlated strictly to antigen density. In WSU-FSCCL, CD74 antigen density is half that of CD20; however, [3H]-thymidine incorporation was inhibited 80% by hLL1 + GAH IgG cross-linking, whereas these cells were unaffected by rituximab + GAH IgG. In addition, Raji cells expressed a higher density

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<th>Table 1. Antigen expression: indirect flow cytometry assay</th>
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<td>CAG</td>
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<td>KMS12-PE</td>
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<td>MC/CAR</td>
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Figure 1. Effects of hLL1 and rituximab on proliferation of cell lines. Antiproliferative effects of the anti-B-cell mAbs were assessed by measuring the uptake of [3H]-thymidine. Cells were cultured with the mAbs with or without a second antibody for cross-linking to mimic the role of effector cells or cross-linking molecules present in vivo. Error bars represent standard deviations (SD). (A) NHL cell lines incubated with hLL1. (B) NHL cell lines incubated with rituximab. (C) MM cell lines incubated with hLL1. (D) MM cell lines incubated with rituximab. *Not determined.
of CD74 than Daudi, SU-DHL-6, and WSU-FSCCL cells, but were less sensitive to inhibition of proliferation by hLL1 + GAH IgG.

Evaluation of the antiproliferative effects of hLL1 in MM cell lines demonstrated that the effectiveness of hLL1 in the presence of GAH second antibody correlated with the presence of antigen in this cell line panel (Figure 1C-D). ARH-77, MC/CAR, CD20⁺, and CD74⁺ cell lines were sensitive to hLL1 and rituximab; CAG, KMS12-PE, CD20⁺, and CD74⁺ were only inhibited by hLL1. The 2 CD20⁺ and CD74⁺ cell lines, ARK and ARD, were insensitive to both mAbs.

Induction of apoptosis

Induction of apoptosis was evaluated by flow cytometry of a panel of CD74⁺ cells. Cells were cultured with the mAbs without a second antibody, with GAM IgG, or with GAH IgG, followed by DNA staining with propidium iodide. (A) Representative histograms are shown for SU-DHL-6 cells. Hypodiploid DNA peaks corresponding to apoptotic nuclei were quantified in region M1. Dashed line indicates no second antibody; solid gray line, GAM IgG; solid black line, GAH IgG. (B) Percentage apoptosis (hypodiploid DNA) induced by cross-linked mAbs is shown for a panel of cell lines. (C) Caspase-3 and -8 activities in Raji cells. Cells were cultured in the presence of hLL1 (5 µg/mL), with both antibodies (LL1 [5 µg/mL] + CL [20 µg/mL]) or no antibody added (Untreated) for 24 hours. Intracellular levels of caspase-3 and -8 were measured by using ApoAlert Caspase assay kits (Clontech).

cross-linked hLL1, approximately 3-fold increases of caspase-3 and caspase-8 activities were detected in Raji cells treated with the cross-linked hLL1 compared with untreated controls or cells treated with hLL1 or with the cross-linking antibody alone (Figure 2C).

The ability of hLL1 to induce ADCC and CMC was assessed using standard ¹⁵¹⁵Cr release assays. In contrast to rituximab, the anti-CD74 mAb did not induce significant levels of ADCC or CMC (Figure 3A-B). This may be related to the rapid internalization of hLL1.

Effects of combining anti-B cell mAbs

Given that combinations of mAbs recognizing different antigens that act by means of distinct mechanisms may enhance antitumor activity, we examined the effects of combining anti-CD74 and anti-CD20 mAbs. Antiproliferative effects of the combination of hLL1 and rituximab were tested and compared with the effects of each mAb given alone, using [³H]-thymidine uptake assays. Results of the combined mAb treatments are shown in Figure 4A-B, with and without cross-linking by GAH second antibody. In all cell lines, the effect of the combination of hLL1, rituximab, and second antibody was equal to or greater than that of the single cross-linked mAb treatments. In 4 of 6 lines (FSCCL, SU-DHL-4, DoHH2, and MC/CAR) growth inhibition of cells given the combination treatment was significantly greater than that of cross-linked rituximab alone. In the other 2 cell lines, Daudi and RL, adding hLL1 to rituximab and GAH did not cause a significant change in proliferation. The effect became more pronounced as the sensitivity to rituximab decreased, except in RL, which is relatively insensitive to hLL1. In all 6 cell lines, the antiproliferative effect of the combined antibody treatment was greater than that of cross-linked hLL1 alone. Statistical significance was reached in all cell lines except FSCCL and MC/CAR, in which the effects of hLL1 alone were most pronounced. In the absence of cross-linking Daudi
was the only tested cell line inhibited by rituximab, and the effect was not enhanced by addition of hLL1. In the absence of GAH second antibody, the combined treatment was more effective than either mAb alone in the more hLL1-sensitive cell lines, FSCCL and MC/CAR; however, statistical significance was not reached (P = .07 and .09, respectively) in the comparison between hLL1 plus rituximab and hLL1 alone.

These studies were also performed using a humanized anti-CD20 mAb, IMMU-106, in place of the human–murine chimeric anti-CD20 mAb rituximab. As shown in Table 2, similar results were obtained with rituximab and IMMU-106 in the in vitro proliferation assays. For example, in the SU-DHL-4 cell line, the anti-CD20 mAbs yielded 8% to 15% inhibition of proliferation compared with untreated cells when given as single agents, and this was increased to between 60% and 65% inhibition when combined with hLL1 treatment. This observation is important because it is expected that in humans, IMMU-106 should be at least as effective as rituximab, and, because of its human framework, it may exhibit different pharmacokinetic, toxicity, and therapy profiles than the chimeric antibody.18

### In vivo effects of hLL1

The therapeutic effects of the anti-CD74 mAb were studied in vivo in SCID mice using 2 NHL cell lines, Raji and Daudi. As shown in Figure 5A, treatment of Raji tumors with hLL1 yielded a significant survival benefit, providing a 45% increase in median survival by either of 2 treatment schedules. In one group, hLL1 was administered 5 times a week for 2 weeks, then twice weekly, at 100 µg; and 112 days, multiple 100–µg doses of hLL1 (5 times a week for 2 weeks, then twice weekly, starting 1 day after cell injection (Figure 5B). The effect of dose escalation was not evaluated. Untreated Daudi-bearing mice had a median survival time of 26.5 days after tumor inoculation compared with 31.5 days in the hLL1-treated animals (P = .0004).

Therapeutic efficacy was found to be markedly greater in the MC/CAR MM cell line. Median survival time in untreated mice was 28 days. Figure 6 shows the results of a study comparing the efficacy of hLL1 in MC/CAR-bearing SCID mice treated by 4 dose schedules. In the experiment shown here, a single injection of hLL1 given 5 days after tumor cell injection yielded a significant survival benefit, providing an approximately 75% increase in median survival time. Survival of MC/CAR-bearing SCID mice treated with a single 350-µg dose of hLL1 was compared with that in mice treated with two 350-µg doses (once a week for 2 weeks), both initiated 5 days after tumor cells were inoculated, and also after multiple 100–µg doses of hLL1 (5 times a week for 2 weeks, then twice weekly), with treatment initiated either 1 day or 5 days after tumor cells were transplanted. Median survival times in the groups in which treatment was initiated 5 days after tumor cells were injected were 49 days, single 350-µg dose; 53 days, 2 doses of 350 µg; and 112 days, multiple 100-µg doses. All 3 survival curves were significantly different than they were for untreated mice (P < .0001), but differences between the groups did not reach statistical significance. Initiation of the multiple 100-µg dose

### Table 2. Effects of combining hLL1 with the chimeric anti-CD20 mAb rituximab and the humanized anti-CD20 MAAb IMMU-106 on proliferation of cell lines

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Relative Proliferation</th>
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<tr>
<td></td>
<td>hLL1</td>
</tr>
<tr>
<td>FSCCL</td>
<td>19.7 ± 6.2</td>
</tr>
<tr>
<td>SU-DHL-4</td>
<td>60.8 ± 3.6</td>
</tr>
<tr>
<td>RL</td>
<td>86.9 ± 4.9</td>
</tr>
<tr>
<td>DoHH2</td>
<td>32.1 ± 3.7</td>
</tr>
<tr>
<td>Daudi</td>
<td>52.2 ± 9.1</td>
</tr>
<tr>
<td>MC/CAR</td>
<td>23.9 ± 3.2</td>
</tr>
</tbody>
</table>

3H-thymidine uptake is shown as a percentage of the uptake in untreated control cells. Results are shown only for incubations performed in the presence of second antibody.

*P < .05 compared to rituximab.
†Compared with IMMU-106.
‡Compared with hLL1 by Student test.

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Figure 5. Survival proportions in SCID mice bearing disseminated NHL cells. (A) mAb hLL1 was administered to Raji-bearing SCID mice at 2 dose schedules, both initiated 1 day after tumor cells were inoculated intravenously. Survival was compared with that in untreated Raji-bearing SCID mice. □, 100 µg/injection, 5 times/wk for 2 weeks, then twice weekly; ○, untreated. (B) Survival of Daudi-bearing SCID mice given a low dose of hLL1 was compared with survival of untreated Daudi-bearing SCID mice. ▲, 25 µg twice-weekly intravenous injections, starting 1 day after injection of tumor cells; •, untreated. Each treatment group contained 10 mice.

Figure 6. Survival proportions in SCID mice bearing disseminated MC/CAR MM cells. mAb hLL1 was administered to MC/CAR-bearing SCID mice at 4 dose schedules. Survival was compared with untreated MC/CAR-bearing SCID mice. □, 350 µg administered 5 days after tumor cells were grafted; ○, 350 µg/injection once a week for 2 weeks, treatment initiated 5 days after tumor cells were injected; ▲, 100 µg/injection 5 times/wk for 2 weeks, then twice weekly, treatment initiated 5 days after tumor cells were inoculated; △, 100 µg/injection 5 times/wk for 2 weeks, then twice weekly, treatment initiated 1 day after tumor cells were injected; and ●, untreated control.

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schedule 1 day after tumor cells were inoculated yielded a median survival time of more than 150 days, with 8 of 10 mice long-term survivors (P = .0021 vs the multiple 100-μg dose schedule initiated 5 days after tumor cells were injected). Thus, the hLL1 survival benefit in myeloma-bearing SCID mice was increased by a multiple-dose schedule and early administration. No treatment-related toxicities were observed, as measured by body weight loss.

Discussion

For patients with hematologic malignancies, mAbs against lineage-specific B-cell antigens have provided clinical benefit.24-26 The chimeric anti-CD20 mAb rituximab induces responses in approximately 50% of patients with low-grade follicular lymphoma, with a median time to progression in responders of 13 months.24 Approximately 40% of initial responders respond to retreatment.25 In patients with more aggressive histologic lymphoma subtypes, rituximab therapy induces an approximately 30% overall response rate.26 However, the rituximab response rate in patients with MM is not as high as that observed for patients with follicular lymphoma. In contrast to the high prevalence of CD20 expression in NHL, only 20% of patients with MM express CD20 on bone marrow plasma cells.27 In a phase 2 clinical study of rituximab in 19 patients with MM, 1 patient had a partial response and 5 patients had stable disease; median time to treatment failure was 5.5 months. Efforts are ongoing to improve on the success of rituximab treatments for B-cell malignancies, including combining mAbs with other biologic agents such as interleukin-2 (IL-2),28 combining mAb therapy with chemotherapeutic agents such as CHOP,29-30 the development of humanized anti-CD20 mAbs,31,32 and the use of mAbs that target antigens other than CD20. Other antibodies in clinical trial for hematologic malignancies include anti-CD22 (epratuzumab),31,32 anti-CD52 (alemtuzumab [Campath-1H]), anti-CD80 (galiximab),34 and anti-HLA-DR (apolizumab [Hu1D10]).35,36 Although these mAbs have shown evidence of antitumor activity in patients, as with rituximab, it is unlikely that these mAbs will be curative as single agents. Thus, the use of additional mAbs and of mAb combinations is important.

In this report, we evaluated the antitumor effects and mechanism of action of hLL1, a humanized anti-CD74 mAb. Because rituximab is widely used for the treatment of B-cell malignancies, the expression of antigen and antitumor effects of hLL1 were compared with those of rituximab. CD74 and CD20 were both expressed on the cell surface of all the NHL cell lines tested; however, CD74 staining intensity was generally lower than that of CD20. In contrast, CD74 was detected on a greater number of MM cell lines than CD20. We demonstrated that, as with rituximab in most human lymphoma or in MM cell lines, hLL1 alone does not show a direct cytotoxic effect in vitro. However, in the presence of an appropriate cross-linking agent, hLL1 causes inhibition of cell proliferation and induces apoptosis. Unlike rituximab, hLL1 induces little or no ADCC or CMC. This is apparently not caused by the IgG constant sequences used in hLL1 because a humanized anti-CD20 mAb, IMMU-106—which has the same IgG1 heavy chain constant regions, hinge, and Cκ as hLL1—mediates ADCC and CMC as effectively as rituximab.18 The absence of ADCC and CMC may be related to the rapid internalization of hLL1. Nevertheless, in SCID mouse xenograft models of NHL and MM, treatment with unconjugated hLL1 yields significant survival benefits, providing greater than a 4.5-fold increase in median survival time in the MM model. Therapeutic efficacy was observed even with the very-low-dose regimens given in the Daudi lymphoma model. Because the antibody has little or no toxicity, we could consider using higher doses or extended dosing to perhaps improve on these results even further. Although cross-linking with GAH second antibody was necessary for hLL1 activity in vitro, both for induction of antiproliferative effects and for apoptosis, GAH was not added in the animal experiments. Thus, physiologic processes are involved in the mechanism of antitumor activity of hLL1 in vivo, and activity was observed without the need for an exogenous cross-linking agent.

The effectiveness of hLL1 in the MC/CAR model of MM was greater than that in the Raji- or Daudi-bearing mice. This effect was seen also in vitro, where the inhibitory effect of hLL1 on DNA synthesis was greatest in MC/CAR. Whether this is because of differences in growth rate, greater sensitivity of MM to the antibody in general compared with that of NHL, or other differences between the cell lines remains to be established. It is apparently not because of the density of cell surface antigen given that Raji cells express a higher level of CD74 than MC/CAR on the cell surface. In a comparison of rituximab and hLL1, we observed that the 2 mAbs differed in their antiproliferative effects on the panel of cell lines examined. The largest differences were observed in the WSU-FSCCL and SU-DHL-6 NHL cell lines and also the CAG MM cell line. SU-DHL-6 cells were more sensitive to rituximab, whereas the WSU-FSCCL and CAG cell lines were more sensitive to the antiproliferative effects of hLL1.

Differences in expression of the complement regulator CD59 have been shown to be associated with resistance to rituximab-mediated CMC in patients with progressive CD20+ MM and Waldenström macroglobulinemia despite rituximab therapy.37 MM and NHL patients also express antigens that may have a role in blocking ADCC, such as Fas ligand, MUC1, or TRAIL.37 Thus, CMC and ADCC regulators are present in various B-cell tumors and are associated with resistance to rituximab. Given that our results indicate that hLL1 activity is apparently unrelated to ADCC and CMC, it may be a useful therapy for patients who are rituximab resistant because of inhibition of the ADCC and CMC pathways.

Thus, several properties emerge that make hLL1 especially attractive as another candidate antibody for the therapy of CD74-expressing malignancies, in particular MM. First, the hLL1 mAb has the ability to affect cell proliferation and to induce apoptosis. Second, CD74 has an appropriate expression profile for a therapeutic target, with high expression on malignant cells, specifically MM, and limited expression on normal tissues. The sensitivity of the MM line in the in vivo model, along with the recent report that CD74 is expressed by most (more than 90%) MM patients compared with only approximately 20% of CD20+ MM patients,3 makes the potential application of the anti-CD74 mAb hLL1 especially promising for the treatment of MM. Third, hLL1 is a good candidate for rituximab-resistant disease, either alone or in combination with other antibodies or agents, because it apparently acts through a distinct mechanism, and the 2 mAbs exhibit different expression and sensitivity profiles on B-cell malignancies. In addition, activity can be augmented when hLL1 is given in combination with an anti-CD20 mAb, either rituximab or IMMU-106.

In addition to its potential use as an unlabeled mAb, the rapid internalization of hLL1 makes it an attractive candidate for further development as a carrier of cytotoxic agents. The rapid internalization of hLL1 can lead to accumulation of large amounts of radioactivity within the cell if the antibody is conjugated to an isotope that remains trapped inside the cell, such as a radiometal. Indeed, radiolabeled hLL1 has been demonstrated to effectively and specifically kill human B-cell lymphoma lines in vitro and in animal models.38-41 Similarly, LL1 has been shown to be extremely effective for drug delivery. A doxorubicin-LL1 conjugate was
found to cure SCID mice bearing advanced human B-lymphoma xenografts with a single low dose of the conjugate.42 The effective use of a doxorubicin–hLL1 conjugate was also shown recently in a MM xenograft model.43

In summary, the hLL1 mAb shows promise as another candidate antibody for the treatment of CD74-expressing malignancies, particularly MM. Application of hLL1 should be examined as single-agent therapy and as part of multiple-agent therapy. Because of its rapid internalization, it also shows considerable promise as a drug immunonjugate or as a carrier of therapeutic radioisotopes. In light of the expression of CD74 in nonhematologic malignancies, the potential of hLL1 as a therapy for these diseases also should be evaluated. The precise mechanism of apoptosis induced by hLL1 is unknown and under evaluation, but the recent identification of CD74 as the cellular receptor for the cytokine MIF suggests involvement in transmembrane signaling pathways.

References


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