

CD74 Is Expressed by Multiple Myeloma and Is a Promising Target for Therapy

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ABSTRACT

Purpose: CD74 (HLA-DR-associated invariant chain) plays a role in antigen presentation. In addition to its expression on antigen-presenting cells, it is expressed by carcinomas of renal, lung, gastric, and thymic origin and by certain sarcomas. The restricted expression of CD74 by normal tissues and its very rapid internalization make CD74 an attractive therapeutic target for both cancer and immunologic diseases. Preclinical efficacy of anti-CD74 monoclonal antibody (mAb) therapy has been demonstrated in B-lymphoma models. Because there are few validated antigenic targets in multiple myeloma, CD74 expression was examined.

Experimental Design: CD74 expression was assessed by immunohistochemistry in bone marrow biopsies of known multiple myeloma cases. Its expression was measured by flow cytometry in multiple myeloma lines, and CD74 mRNA expression was determined by reverse transcription-PCR. In addition, the *in vitro* antiproliferative effect of LL1 mAb was evaluated on a CD74+ multiple myeloma cell line using a [³H]thymidine incorporation assay.

Results: CD74 expression was observed in 19 of 22 cases of multiple myeloma, with most expressing moderate to high levels in the majority of malignant plasma cells. CD74 was expressed by most multiple myeloma cell lines, as was CD74 mRNA, at levels mirroring CD74 protein. Also, unlabeled LL1 mAb mediated *in vitro* growth inhibition of a CD74+ multiple myeloma cell line.

Conclusions: CD74 expression is frequent in multiple myeloma, with predominant expression by the malignant plasma cells. Because anti-CD74 mAbs internalize very rapidly and LL1 mAb has shown efficacy in B-lymphoma models, CD74 represents a novel and promising target for treat-

ment of multiple myeloma. Therefore, LL1 mAb is well suited as a carrier of radionuclides, drugs, or toxins, and also has activity as an unlabeled mAb, thereby supporting its development for this unmet need in cancer therapy.

INTRODUCTION

CD74 was originally identified as the invariant chain that is associated with the α and β chains of HLA-DR (MHC class II). CD74 (invariant chain) is a type-II transmembrane protein, with 30 NH₂-terminal, intracytoplasmic amino acid residues, a membrane-spanning segment from residues 31 to 55, and an extracytoplasmic domain consisting of 160 amino acids (1, 2). Once synthesized, CD74, DR α , and DR β begin to associate within the endoplasmic reticulum. This is believed to occur by the sequential addition of DR α / β heterodimers to a trimeric core of CD74 molecules until a nine-subunit complex with equimolar amounts of these three chains is formed (3, 4). DR-CD74 complexes then progressively traffic to the late endosomal compartment where CD74 is cleaved into peptide fragments by endosomal/lysosomal proteases. These CD74 cleavage fragments have reduced affinity for DR and allow for the displacement of CD74 by cognate antigenic peptides. In the trafficking of newly synthesized DR-CD74 complexes to and from the late endosomal compartment, a sizable pool resides transiently on the cell surface. This very dynamic, plasma membrane DR-CD74 pool internalizes rapidly to the endosome (a process that is promoted by sequence motifs in the cytoplasmic tail of CD74) and is replenished by newly synthesized DR-CD74 complexes. Thus, upon binding CD74 on the cell surface, anti-CD74 monoclonal antibodies (mAbs) become rapidly cointernalized. This was initially observed on cells with known antigen-presenting capacity, but later was demonstrated in a wider range of malignant cell lines, including those derived from epithelial cancers (5, 6). In some circumstances, however, CD74 may be expressed on the cell surface in the absence of class II, as was shown in a mutant, class II-negative cell line (7).

During the initial evaluation of the LL1 mAb, several properties emerged that made it a candidate for additional development: its very rapid internalization (an intrinsic property of CD74); its expression by a range of cancer cell lines; and its restricted expression by normal tissues. These findings pointed to the strong potential of targeting the CD74 antigen for imaging and therapy of cancer (8). Indeed, preclinical studies demonstrated the *in vitro* cytotoxicity of radionuclide-conjugated anti-CD74 mAb (LL1) for various cancer cell lines, as well as the major therapeutic effect of this radioantibody in disseminated B-lymphoma murine xenograft models (9–11). In addition, a doxorubicin conjugate of LL1 was shown recently to have significant antitumor efficacy in a disseminated B-lymphoma xenograft model, with cures being observed in the vast majority of LL1-doxorubicin-treated mice given a single, submaximal dose of this immunoconjugate (12). Because multiple myeloma

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is a cancer of B-cell lineage, yet expresses few early or late B-cell-specific antigens (such as CD19, 20, and 22), we evaluated the expression of CD74 in multiple myeloma clinical specimens and derivative cell lines. CD74 was found to be expressed by the vast majority of multiple myeloma clinical specimens and by a majority of multiple myeloma cell lines, and this correlated with *CD74* mRNA expression in the multiple myeloma cell lines. Also, the LL1 mAb was shown to mediate *in vitro* growth inhibition of a multiple myeloma line.

MATERIALS AND METHODS

Monoclonal Antibodies. The LL1 mAb is a murine IgG1 that was kindly provided by Immunomedics, Inc. (Morris Plains, NJ). This mAb has been characterized previously and has been shown to be reactive with an extracellular domain of CD74 (5). LL1 mAb has been humanized according to an approach used for three other mAbs (13–15). It has been shown to retain the binding characteristics of the original murine mAb (16). To confirm results obtained with the LL1 mAb, another widely studied anti-CD74 mAb, LN2 (Biogenex, San Ramon, CA), was used. HLA-DR expression was assessed by immunohistochemistry using the TAL.1B5 mAb (DAKO, Carpinteria, CA). For the measurement of HLA-DR expression by flow cytometry, the L243 mAb was obtained (American Type Culture Collection, Manassas, VA). To confirm the identity of malignant plasma cells, the anti-CD138/syndecan-1 mAb, B-B4 (Serotec, Oxford, United Kingdom), was used. The negative control mAb that was used for both flow cytometry and immunohistochemistry was a nonbinding murine IgG1 myeloma protein, Ag8 (P3 × 63Ag8; obtained from the American Type Culture Collection).

Clinical Specimens. Archived, paraffin-embedded blocks from bone marrow trephine biopsies that had been fixed in Bouin's solution were used to determine the expression of CD74 as described previously (17). Five- μ m sections were cut and mounted on 3-aminopropyltriethoxy-silane-coated slides, dried, and deparaffinized before immunohistochemistry. Immunohistochemistry was performed by indirect immunoperoxidase staining of tissue sections using a TechMate500 BioTek automated immunostainer (Ventana Medical Systems, Tucson, AZ). In preparation for immunostaining, after the final hydration step with alcohol, the Bouin's-fixed slides were placed in 70% EtOH with lithium carbonate to remove any remaining fixative. Endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide. Malignant plasma cells were identified by morphologic assessment and by immunohistochemistry using an anti-CD138/syndecan-1 antibody (B-B4; Serotec, Oxford, United Kingdom). CD74 expression was detected using the LL1 mAb, and these results were confirmed with a second anti-CD74 mAb, LN2.

Cell Lines. The multiple myeloma cell lines, U266B1, NCI-H929, RPMI 8226, and MC/CAR, as well as the B-lymphoma lines, Raji and Ramos, were obtained from the American Type Culture Collection. Two other multiple myeloma cell lines, KMS12-PE and KMS12-BM, were generously provided by Dr. Takemi Otsuki (Kawasaki Medical School, Okayama, Japan). Additional multiple myeloma lines, ARK, ARP-1, ARD, and CAG, were generously provided by Dr.

Joshua Epstein (University of Arkansas, Little Rock, AK). The multiple myeloma lines, OPM-2 and OPM-6, were kindly supplied by Dr. Kenji Oritani (Osaka University, Osaka, Japan), and the MM.1R line was a gift from Dr. Steven Rosen (Northwestern University, Chicago, IL). All of the cell lines were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum plus antibiotics, except for the MC/CAR line, for which the media preparation recommended by American Type Culture Collection was used.

Flow Cytometry. For the measurement of cell surface CD74, cell lines were washed and set up at $\sim 0.5 \times 10^6$ cells/tube and incubated with 10 μ g/mL of control and test mAbs for 30 minutes at 4°C in flow cytometry buffer (PBS/3% fetal bovine serum/0.1% NaN₃). After washing twice with the same buffer, the second step reagent, pretitered (typically 1:40 dilution in flow cytometry buffer) F(ab')₂ goat antimouse IgG-FITC (Biosource International, San Diego, CA), was added, and cells were incubated for an additional 30 minutes at 4°C. After two washes in flow cytometry buffer, the cells were then fixed with 1.5% paraformaldehyde in PBS and analyzed on a FAC-SCaliber flow cytometer (Becton-Dickinson, Mountain View, CA). A minimum of 5,000 events were analyzed. To measure intracellular CD74, cell lines were washed twice in PBS and then were fixed in PBS with 0.2% paraformaldehyde for 1 hour at 4°C. Cells were pelleted and resuspended in PBS/0.2% Tween 20 for staining with both first and second antibodies. The same panel of mAbs was used, and the staining procedure was the same as described above, with PBS/0.2% Tween 20 being used as the incubation and washing buffer.

Reverse Transcription-PCR. The starting material for reverse transcription-PCR (RT-PCR) was total RNA, which was isolated from PBS-washed cells that were solubilized with a guanidine isothiocyanate-based buffer (Tri-Reagent, Sigma-Aldrich, St. Louis, MO), according to a modification of the method of Chomczynski (18). Total RNA (5 μ g) was used as the template for cDNA synthesis, using the First Strand kit of Novagen (Madison, WI) according to the manufacturer's instructions, with 5% of the resulting cDNA product being used for each PCR reaction. For PCR, primers and deoxynucleotide triphosphates were added at concentrations of 0.5 μ mol/L and 200 μ mol/L, respectively. The CD74 primers TGA-CCA-GCG-CGA-CCT-TAT-CT (forward) and GAG-CAG-GTG-CAT-CAC-ATG-GT (reverse) were used, which amplify a 384-bp sequence in the CD74 5' coding region (GenBank accession no. X00497, human HLA-DR-associated invariant chain). Thermostable DNA polymerase (1.25 units of RedTaq; Sigma-Aldrich) was added to each tube, and 27 or 35 cycles of PCR were carried out under the following conditions: initial denaturation of 94°C × 5 minutes, annealing at 54°C × 90 seconds, extension at 72°C × 1 minute, and denaturation at 94°C × 1 minute followed by terminal extension at 72°C × 10 minutes. Thirty-five cycles of PCR were used to assess samples that were negative after 27 cycles.

Cellular Proliferation Assay. The *in vitro* effects of murine LL1, humanized LL1, and control antibodies were assessed in cell growth assays in 96-well, flat-bottomed plates, in which 1.5 to 3 × 10³ cells/well were added to triplicate wells. MAbs were added at a concentration of 5 μ g/mL, and cross-linking second antibody (goat antimouse F_c or goat antihuman

F₀; The Jackson Laboratory, West Grove, PA) were added at 20 µg/mL in a final volume of 200 µL of standard growth medium (RPMI 1640 supplemented with 10% fetal bovine serum and antibiotics) per well. The plates were then incubated at 37°C in a 5% CO₂-supplemented atmosphere for 48 hours, at which point 0.5 µCi/well of [³H]thymidine (NEN, Billerica, MA) was added. The plates were then incubated for an additional 24 hours, at which time they were harvested onto glass fiber filters. Scintillation fluid was added to the filters, and the radioactivity was counted in a β counter (MicroBeta, Wallac/Perkin-Elmer, Boston, MA). Growth inhibition was calculated using the formula: % of control (untreated cells) = (counts/min_{test}/counts/min_{control}) × 100.

RESULTS

CD74 Is Expressed by Malignant Multiple Myeloma Plasma Cells. A representative group of 22 multiple myeloma trephine bone marrow biopsies was analyzed for CD74 expression by immunohistochemistry using the LL1 mAb. Nineteen of these 22 specimens (86.4%) exhibited positive staining (*i.e.*, ≥50% of malignant plasma cell present), with 16 of the 19 cases showing CD74 staining in >95% of multiple myeloma plasma cells. Malignant plasma cells from adjacent histologic sections were identified by morphology and immunohistochemistry with an anti-CD138 mAb. Of the 19 positive cases, 7 exhibited cellular staining intensity of 2 to 3+ with the LL1 mAb. Only 3 cases showed 1 to 2+ staining intensity, with the remaining 9 cases showing staining that ranged from 1 to 3+ across the malignant plasma cell population. This group of multiple myeloma cases was also assessed for HLA-DR expression. In contrast to most normal and malignant cell types, which coexpress DR and CD74, only 1 of the 22 cases was DR+. This finding may have important implications for the antigenicity of multiple myeloma cells with respect to their recognition by the host immune system. To confirm the results with the LL1 mAb, 8 CD74+ multiple myeloma cases from this group were also stained with the LN2 mAb. This anti-CD74 has been widely used for immunohistochemical detection of CD74. All 8 of these LL1-positive multiple myeloma cases also showed positive staining with the LN2 mAb (Fig. 1).

CD74 Is Expressed by Multiple Myeloma Cell Lines.

A panel of 14 multiple myeloma lines was analyzed by flow cytometry. Positive cell surface CD74 expression as assessed by LL1 mAb staining was detected in 9 of 14 cell lines (64.3%). In contrast to the clinical multiple myeloma specimens, HLA-DR was coexpressed by 8 of the 9 CD74+ multiple myeloma cell lines, with 1 cell line showing a low level of CD74 expression without any detectable DR expression. To determine the expression of total cellular CD74, flow cytometry was performed on fixed and permeabilized cell lines with the two anti-CD74 mAbs, LL1 and LN2. There was good agreement in the levels of total cellular CD74 expression in multiple myeloma lines between the two anti-CD74 mAbs, with only minor differences noted in cell lines that expressed low levels of CD74 (negative or 1+). Table 1 shows the results of flow cytometric analysis of fixed permeabilized multiple myeloma and control cell lines stained with mLL1 and categorized according to their expression levels. The positive control cell lines for flow cytometry,

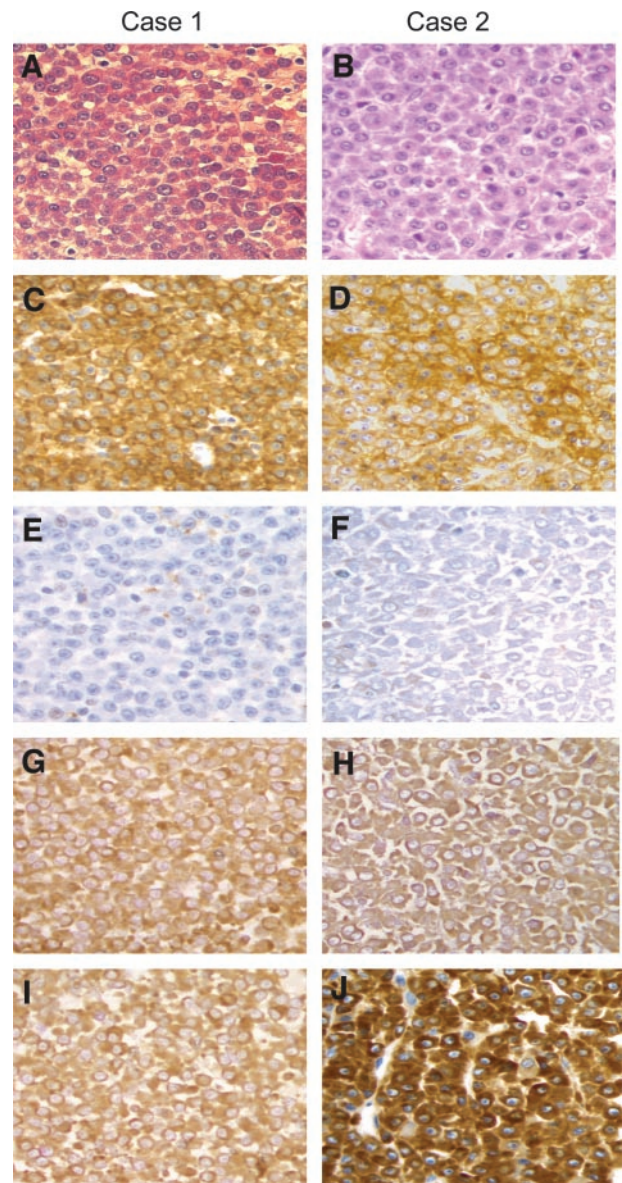


Fig. 1 Immunohistochemical staining of trephine bone marrow biopsies. Photomicrographs stained with H&E and immunohistochemical staining of representative, adjacent sections of trephine bone marrow biopsies of 2 of the 19 CD74+ MM cases are shown. *A* and *B* are stained with H&E; *C* and *D* are stained with the anti-CD138 mAb, B-B4; *E* and *F* are stained with a nonbinding murine mAb, Ag8; *G* and *H* are stained with the anti-CD74 mAb, LL1; *I* and *J* show staining with another anti-CD74 mAb, LN2.

RT-PCR, and the *in vitro* growth inhibition assays were the B-lymphoma cell lines, Raji and Ramos. Other cancer cell lines were analyzed in these experiments (data not shown) as well as in a previous study (6). In current and previous studies, human carcinoma cell lines of lung, renal, colon, and ovarian origin showed infrequent, low-level expression of CD74, whereas some melanoma cell lines exhibited moderate levels of expression.

Table 1 Flow cytometric analysis of total cellular CD74 expression in MM and control cell lines

	LL1 reactivity
CAG	+++ (96.6 ± 0.3)
RPMI 8226	+++ (59.8 ± 3.9)
MM1.R	++ (46.0 ± 2.7)
KMS11	++ (32.7 ± 1.1)
KMS12-PE	+++ (78.3 ± 4.2)
KMS12-BM	++ (43.1 ± 4.7)
U266B1	++ (48.0 ± 4.8)
MC/CAR	+++ (82.3 ± 3.8)
NCI-H929	- (0.6 ± 1.0)
OPM-2	- (0.9 ± 1.3)
OPM-6	- (0.3 ± 1.2)
ARD	- (0.8 ± 1.0)
ARK	+ (6.6 ± 2.4)
ARP-1	- (1.2 ± 1.4)
Raji (B-lymphoma)	+++ (80.2 ± 2.1)
Ramos (B-lymphoma)	+++ (81.9 ± 3.7)

NOTE. The categorized and corresponding numerical values (in parentheses ± SD) above represent the mean of three flow cytometry assays using the mLL1 mAb on permeabilized MM and control lines. The percent positive value was defined as total positive event rate minus the background level of the negative control mAb, Ag8. -, <5% positive cells; +, 5–20% positive cells; ++, 20–50% positive cells; +++, >50% positive cells.

CD74 mRNA Is Expressed by Multiple Myeloma Lines in Relation to CD74 Protein Expression. Because there is a substantial intracellular pool of CD74, the best assessment of its expression is by assays that measure its total cellular content, such as immunohistochemistry or flow cytometry using permeabilized cells. When near-maximal RT-PCR was carried out (35 cycles), 13 of the 14 multiple myeloma lines in the panel had a discernible CD74 signal. When submaximal (linear range) PCR amplification was used (27 cycles) on this entire panel, 3 multiple myeloma lines were negative and 3 had a faint CD74 signal, which is exemplified in one representative analytical agarose gel (Fig. 2). This panel of multiple myeloma lines showed a concordant grouping of CD74 mRNA expression with CD74 protein expression in that the 6 multiple myeloma lines with a negative or low-level PCR signal (3 negative and 3 low) were also the lowest 6 CD74-expressing lines by flow cytometry (Table 1; 5 negative; one 1+). The other 8 multiple myeloma lines that were 2 to 3+ by flow cytometry showed moderate to high signal intensity by RT-PCR. In all of the RT-PCR experiments, the CD74 amplicon migrated consistently as a single band at the appropriate molecular size (384 bp). These results served to confirm the expression of CD74 that was observed in the clinical multiple myeloma specimens stained with two different anti-CD74 mAbs.

The Humanized Form of the LL1 mAb (hLL1) Inhibits *In vitro* Proliferation of Both B-Lymphoma and Multiple Myeloma Cell Lines. Previous experiments with the original murine form of LL1, mLL1, as well as the humanized form, hLL1, showed *in vitro* growth-inhibitory activity for B-lymphoma cell lines. This was associated with specific apoptotic events, such as caspase activation and DNA fragmentation (16). The growth-inhibitory activity of mLL1 and hLL1 was dependent on the addition of a specific cross-linking antibody, goat antimouse F_c or goat antihuman F_c, respectively. Similar *in vitro*

experiments using hLL1 also showed significant growth inhibitory activity in 1 of the multiple myeloma cell lines (Fig. 3), with this multiple myeloma line (MC/CAR) exhibiting somewhat greater inhibition than a control B-lymphoma line. The multiple myeloma line, ARK, which has no detectable surface CD74 and only 1+ intracellular CD74, did not show hLL1-mediated growth inhibition. These initial results have been confirmed with 2 other CD74+ multiple myeloma lines, both of

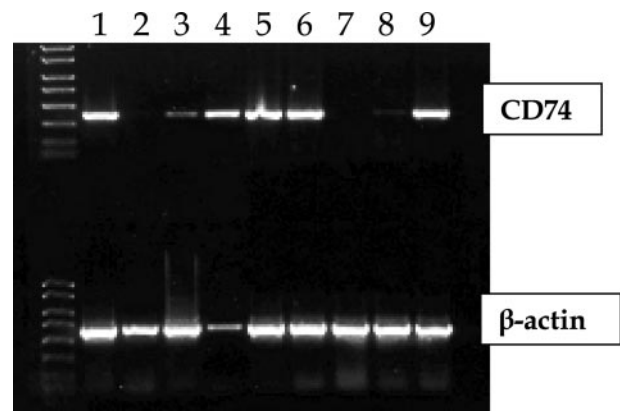


Fig. 2 CD74 mRNA expression by RT-PCR. An ethidium-bromide stained agarose gel is shown on which equal aliquots of RT-PCR reactions were loaded. Submaximal amplification (27 cycles) of cDNA from a representative subset of the panel of MM lines as well as a positive control, B-lymphoma line (Ramos) was performed for CD74 (top) and β-actin (bottom). On the (far left) are MW markers, which indicate that the molecular size of the CD74 amplicon is consistent with that predicted from the published DNA sequence. The samples were loaded onto lane numbers as follows: 1, Raji; 2, NCI-H929; 3, MC/CAR; 4, MM1.R; 5, U266B1; 6, CAG; 7, OPM-2; 8, ARK; and 9, ARP-1.

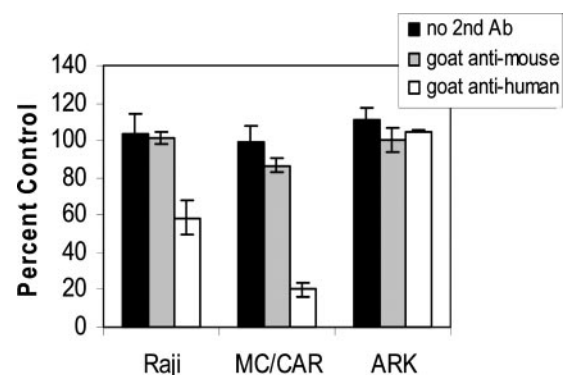


Fig. 3 *In vitro* treatment of cell lines with LL1 mAb. The results of replicate *in vitro* cell proliferation assays using [³H]thymidine uptake as the readout are shown. The Raji (B-lymphoma) cell line served as a control and comparator for the MM line, MC/CAR. The MM line, ARK, was included as a negative control, because it expresses no detectable cell surface CD74. The mean [³H]thymidine uptake value for the cell lines in wells containing only growth medium was defined as 100% control level. The first antibody, hLL1, was added to wells at 5 μg/mL, followed by either medium or one of the 2nd antibodies, goat anti-mouse F_c (nonspecific) or goat antihuman F_c (specific), at a concentration of 20 μg/mL.

which showed hLL1-mediated growth inhibition (16). This indicates that multiple myeloma lines, like a variety of non-Hodgkin's lymphoma (NHL) cell lines, also show sensitivity to treatment with unlabeled anti-CD74 mAbs. These data provide a basis for ongoing *in vivo* testing, both preclinically and clinically, in multiple myeloma, and indicate that there may be an important unlabeled or naked antibody effect of hLL1 that could augment the effects of hLL1-conjugates, as has been noted with anti-CD20 mAbs and their radioconjugates.

DISCUSSION

This report is the first, of which we are aware, to document CD74 expression in multiple myeloma. A previous report suggested that CD74 expression was highest in NHL cases corresponding to the midpoint of the normal B-cell differentiation pathway (diffuse large B-cell NHL), with lower CD74 mRNA levels in more differentiated cancers, such as immunoblastic NHL or myeloma (19). These authors, however, did not measure CD74 protein levels. Several other studies of CD74 protein expression have demonstrated its expression across a range of B-cell malignancies, as well as by the Reed-Sternberg cell of Hodgkin's lymphoma (20–23). Similar results in a broad group of NHL cases (indolent and aggressive histologies) were reported previously with the LL1 mAb; 55 of 60 NHL cases were positive by immunohistochemistry with the LL1 mAb, as were 4 of 5 cases of Hodgkin's lymphoma (8).

In studies using cell fusion between human B cells and mouse plasmacytoma lines, a down-regulation of both HLA-DR and CD74 expression was observed (24). These data together with the prior data on CD74 mRNA expression in B-cell cancers led to the notion that CD74 expression is down-regulated with differentiation toward plasma cells, as is the case with other B-cell-associated CD molecules. Our finding that CD74 is strongly expressed by the vast majority of multiple myeloma cases has important therapeutic implications, because the B-cell-associated antigens, CD19, CD20, and CD22, are expressed on only a small percentage of multiple myeloma cases. Also, CD74 is a novel and promising therapeutic target, because it has restricted normal tissue expression (8). In addition, both radiolabeled and doxorubicin-conjugated forms of the anti-CD74 mAb, LL1, have been shown to have significant *in vitro* cytotoxicity for NHL and other cell lines, as well as strong therapeutic efficacy in mouse xenograft models of NHL (9–12). The activity of the LL1-doxorubicin conjugate was striking in that it was curative in >80% of B-lymphoma-bearing mice that received a single dose of this conjugate at either 5 or 10 days after intravenous injection of B-lymphoma cells; this effect occurred at a dose that was less than one-third of the maximum tolerated dose of this conjugate (12). It is also noteworthy that targeting CD74 with low-energy, Auger electron-emitting radioconjugates results in strong therapeutic effects in these same NHL models (11). Preclinical studies with mAbs labeled with Auger-emitters (e.g., ^{125}I , ^{111}In , ^{67}Ga) have shown that these radioconjugates have significantly less toxicity than β -emitting (e.g., ^{131}I , ^{90}Y) conjugates but equivalent or greater efficacy in lymphoma and solid tumor models (11, 25). Phase I and II clinical trials using ^{111}In -labeled somatostatin receptor-binding peptides in patients with advanced neuroendocrine tumors have

demonstrated the feasibility, safety, and antitumor activity of targeted Auger-emitter therapy (26, 27).

Because CD74 expression was only seen on some small lymphocytes and histiocytic cells and not on bone marrow precursor cells, it is a logical antigenic target for malignancies involving the bone marrow or reticuloendothelial system. Thus, a pilot clinical imaging study was conducted previously to evaluate the imaging/targeting potential of a $^{99\text{m}}\text{Tc}$ -labeled Fab' fragment of LL1. In the 6 patients with various malignancies, including 1 patient with multiple myeloma and 2 with NHL, this mAb was shown to be safe and to provide rapid, effective targeting of the bone marrow and spleen (28). In addition, cell surface CD74 expression can be up-regulated on various cancer cell lines using IFN- γ (6), a clinically available cytokine, thereby providing an opportunity to additionally enhance tumor cell targeting with the LL1 mAb.

It is also of interest that the LL1 mAb shows *in vitro* cytotoxicity for B-lymphoma and multiple myeloma cell lines as an unlabeled mAb. Because the primary function of CD74 was thought to be a chaperone-like molecule that promoted antigen presentation by HLA-DR, it was not anticipated that an anti-CD74 mAb (LL1) would possess growth-inhibitory function. Recently, however, CD74 was shown to be the cellular receptor for the cytokine MIF (29). Addition of MIF to various CD74+ cell types (including macrophages, a B-lymphoma, and a fibroblast cell line) led to signaling events such as phosphorylation of extracellular regulated kinase-1 and -2. Addition of MIF to a B-lymphoma cell line induced a positive proliferative response that was blocked by the anti-CD74 mAb, LN2 (5). Whether mAbs, such as LL1, mediate their growth-inhibitory activity by blocking the effects of stimulatory growth factors, such as MIF, remains to be determined.

Finally, it should be noted that the expression of CD74 by malignant cells may have implications for immunologic escape. In a syngeneic mouse model, the aggressive, SaI (HLA class II-/CD74-) sarcoma was rendered immunogenic and less aggressive by transfection of HLA class II (DR equivalent). It was then shown that the aggressive *in vivo* behavior of this cell line could be restored by increasing CD74 levels by transfection (30). An association between pathological dedifferentiation, aggressive clinical behavior, and CD74 expression also has been observed in colon cancer specimens (31). The CD74+/DR- phenotype observed in the multiple myeloma cases we studied may have similar implications for the biology of multiple myeloma.

In conclusion, we have demonstrated that CD74 is expressed by the vast majority of multiple myeloma cases. The potential of the anti-CD74 mAb LL1 to target malignant cells in the bone marrow, as well as the *in vitro* and preclinical *in vivo* antitumor effects of LL1-drug and radioconjugates, make it a promising therapeutic agent for multiple myeloma, as well as other CD74+ malignancies.

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REFERENCES

- Claesson L, Larhammer D, Rask L, Peterson PA. cDNA clone for the human invariant gamma chain of class II histocompatibility antigens and its implications for the protein structure. *Proc Natl Acad Sci USA* 1983;80:7395–9.
- Strubin M, Mach B, Long EO. The complete sequence of the mRNA for the HLA-DR-associated invariant chain reveals a polypeptide with an unusual transmembrane polarity. *EMBO J* 1984;3:869–72.
- Roche PA, Marks MS, Cresswell P. Formation of a nine-subunit complex by HLA class II glycoproteins and the invariant chain. *Nature* 1991;354:392–4.
- Lamb CA, Cresswell P. Assembly and transport properties of invariant chain trimers and HLA-DR-invariant chain complexes. *J Immunol* 1992;148:3478–82.
- Hansen HJ, Ong GL, Diril H, et al. Internalization and catabolism of radiolabeled antibodies to the MHC class II invariant chain by B-cell lymphomas. *Biochem J* 1996;320:293–300.
- Ong GL, Goldenberg DM, Hansen HJ, Mattes MJ. Cell surface expression and metabolism of major histocompatibility complex class II invariant chain (CD74) by diverse cell lines. *Immunology* 1999;98:296–302.
- Henne C, Schwenk F, Koch N, Moller P. Surface expression of the invariant chain (CD74) is independent of concomitant expression of major histocompatibility complex class II antigens. *Immunology* 1995;84:177–82.
- Pawlak-Byczkowska EJ, Hansen HJ, Dion AS, Goldenberg DM. Two new monoclonal antibodies, EPB-1 and EPB-2, reactive with human lymphoma. *Cancer Res* 1989;49:4568–77.
- Griffiths GL, Govindan SV, Sgouros G, Ong GL, Goldenberg DM, Mattes MJ. Cytotoxicity with Auger electron-emitting radionuclides delivered by antibodies. *Int J Cancer* 1999;81:985–92.
- Ong GL, Elsamra SE, Goldenberg DM, Mattes MJ. Single-cell cytotoxicity with radiolabeled antibodies. *Clin Cancer Res* 2001;7:192–201.
- Ochakovskaya R, Osorio L, Goldenberg DM, Mattes MJ. Therapy of disseminated B-cell lymphoma xenografts in SCID mice with an anti-CD74 antibody conjugated with ^{111}In , ^{67}Ga , or ^{90}Y . *Clin Cancer Res* 2001;7:1505–10.
- Griffiths GL, Mattes MJ, Stein R, et al. Cure of SCID mice bearing human B-lymphoma xenografts by an anti-CD74 antibody-anthracycline drug conjugate. *Clin Cancer Res* 2003;9:6567–71.
- Leung SO, Goldenberg DM, Dion AS, et al. Construction and characterization of a humanized, internalizing, B-cell (CD22)-specific, leukemia/lymphoma antibody, LL2. *Molecular Immunol* 1995;32:1416–27.
- Govindan SV, Stein R, Qu Z, et al. Preclinical therapy of breast cancer with a radioiodinated humanized anti-EGP-1 monoclonal antibody: advantage of a residualizing iodine radiolabel. *Breast Cancer Res Treat* 2004;84:173–82.
- Stein R, Qu Z, Chen S, et al. Characterization of a new humanized anti-CD20 monoclonal antibody, IMMU-106, and its use in combination with the humanized anti-CD22 antibody, epratuzumab, for the therapy of non-Hodgkin's lymphoma. *Clin Cancer Res* 2004;10:2868–78.
- Stein R, Qu Z, Cardillo TM, et al. Anti-proliferative activity of a humanized anti-CD74 monoclonal antibody, hLL1, on B-cell malignancies. *Blood* in press 2004.
- Ely SA, Knowles DM. Expression of CD56/neural cell adhesion molecule correlates with the presence of lytic bone lesions in multiple myeloma and distinguishes myeloma from monoclonal gammopathy of undetermined significance and lymphomas with plasmacytoid differentiation. *Am J Pathol* 2002;160:1293–99.
- Chomczynski P, Mackey K. Substitution of chloroform by bromochloropropane in the single-step method of RNA isolation. *Anal Biochem* 1995;225:163–4.
- Gondo H, Narni F, Lee MS, Blick MB, Cabanillas F, Saunders GF. HLA class II antigen associated invariant chain gene expression in malignant lymphoma. *Br J Hematol* 1987;67:413–7.
- Carbone A, Pinto A, Gloghini A, Volpe R, Zagonel V. B-zone small lymphocytic lymphoma: a morphologic, immunophenotypic, and clinical study with comparison to “well-differentiated” lymphocytic disorders. *Hum Pathol* 1992;23:438–48.
- Stroup R, Sheibani K. Antigenic phenotypes of hairy cell leukemia and monocytoid B-cell lymphoma: an immunohistochemical evaluation of 66 cases. *Hum Pathol* 1992;23:172–7.
- Bertero M, Novelli M, Fierro MT, Bernengo MG. Mantle zone lymphoma: an immunohistologic study of skin lesions. *J Am Acad Dermatol* 1994;30:23–30.
- Sarker AB, Akagi T, Jeon HJ, et al. Bauhinia purpurea—a new paraffin section marker for Reed-Sternberg cells of Hodgkin's disease. A comparison with Leu-M1 (CD15), LN2 (CD74), peanut agglutinin, and Ber-H2 (CD30). *Am J Pathol* 1992;141:19–23.
- Venkitaraman AR, Culbert EJ, Feldmann M. A phenotypically dominant regulatory mechanism suppresses major histocompatibility complex class II gene expression in a murine plasmacytoma. *Eur J Immunol* 1987;17:1441–6.
- Behr TM, Sgouros G, Vougiokas V, et al. Therapeutic efficacy and dose-limiting toxicity of Auger-electron vs. beta emitters in radioimmunotherapy with internalizing antibodies: evaluation of ^{125}I - vs. ^{131}I -labeled CO17–1A in a human colorectal cancer model. *Int J Cancer* 1998;76:738–48.
- Anthony LB, Woltering EA, Espenan GD, Cronin MD, Maloney TJ, McCarthy KE. Indium-111-pentetreotide prolongs survival in gastroenteropancreatic malignancies. *Semin Nucl Med* 2002;32:123–32.
- De Jong M, Valkema R, Jamar F, et al. Somatostatin receptor-targeted radionuclide therapy of tumors: preclinical and clinical findings. *Semin Nucl Med* 2002;32:133–40.
- Juweid M, Dunn RM, Sharkey RM, Rubin AD, Hansen HJ, Goldenberg DM. $^{99\text{m}}\text{Tc}$ -LL1. A potential new bone marrow imaging agent. *Nucl Med Commun* 1997;18:142–8.
- Leng L, Metz CN, Fang Y, et al. MIF signal transduction initiated by binding to CD74. *J Exp Med* 2003;197:1467–76.
- Clements VK, Baskar S, Armstrong TD, Ostrand-Rosenberg S. Invariant chain alters the malignant phenotype of MHC class II+ tumor cells. *J Immunol* 1992;149:2391–96.
- Jiang Z, Xu M, Savas L, LeClair P, Banner BF. Invariant chain expression in colon neoplasms. *Virchows Arch* 1999;435:32–6.