

# Drug-Resistant AML Cells and Primary AML Specimens Are Killed by $^{111}\text{In}$ -Anti-CD33 Monoclonal Antibodies Modified with Nuclear Localizing Peptide Sequences

Veerle Kersemans<sup>1</sup>, Bart Cornelissen<sup>1</sup>, Mark D. Minden<sup>2,3</sup>, Joseph Brandwein<sup>2</sup>, and Raymond M. Reilly<sup>1,4,5</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, University of Toronto, Toronto, Ontario, Canada; <sup>2</sup>Department of Hematology and Oncology, University Health Network, University of Toronto, Toronto, Ontario, Canada; <sup>3</sup>Division of Stem Cell and Developmental Biology, Ontario Cancer Institute, Ontario, Canada; <sup>4</sup>Department of Medical Imaging, University of Toronto, Toronto, Ontario, Canada; and <sup>5</sup>Division of Pathophysiology and Clinical Investigation, Toronto General Research Institute, Toronto, Ontario, Canada

Multidrug resistance (MDR) is a major challenge to the successful treatment of acute myeloid leukemia (AML). Our purpose was to determine whether  $^{111}\text{In}$ -HuM195 anti-CD33 antibodies modified with peptides harboring nuclear localizing sequences (NLS) could kill drug-resistant AML cell lines and primary AML patient specimens expressing MDR transporters through the emission of Auger electrons. **Methods:** HuM195, M195, and irrelevant mouse IgG (mIgG) were conjugated to  $10 \pm 3$  NLS peptides and then labeled with  $^{111}\text{In}$  by diethylenetriaminepentaacetic acid substitution to a specific activity of 1–8 MBq/ $\mu\text{g}$ . The binding affinity of HuM195 and M195 was determined for HL-60 and mitoxantrone-resistant HL-60-MX-1 cells. Nuclear localization of  $^{111}\text{In}$ -NLS-HuM195,  $^{111}\text{In}$ -NLS-M195,  $^{111}\text{In}$ -HuM195, and  $^{111}\text{In}$ -M195 was measured by subcellular fractionation. The antiproliferative effects of  $^{111}\text{In}$ -NLS-HuM195,  $^{111}\text{In}$ -NLS-M195,  $^{111}\text{In}$ -HuM195, and  $^{111}\text{In}$ -M195 (2.5–250 kBq/well) on HL-60 and HL-60-MX-1 were studied using the WST-1 assay. Clonogenic survival of HL-60 and HL-60-MX-1 leukemic cells and 10 primary AML specimens with MDR phenotype (assessed by flow cytometry) was determined after exposure for 3 h at 37°C to 2.5–250 mBq/cell of  $^{111}\text{In}$ -NLS-HuM195,  $^{111}\text{In}$ -HuM195, or  $^{111}\text{In}$ -NLS-mIgG. Clonogenic survival versus the amount of radioactivity incubated with the cells (mBq/cell) was plotted, and the mean lethal amount of radioactivity and the lower asymptote of the curve (plateau) were determined. **Results:** The  $^{111}\text{In}$ -labeled anti-CD33 monoclonal antibodies HuM195 and M195 modified with NLS were efficiently routed to the nucleus of HL-60 cells and their mitoxantrone-resistant clone after CD33-mediated internalization. The following are the principal findings of our study:  $^{111}\text{In}$ -NLS-HuM195 was more effective at killing HL-60 and HL-60-MX-1 cells than was  $^{111}\text{In}$ -HuM195, a strong correlation between the specific activity of the  $^{111}\text{In}$ -labeled radioimmunoconjugates and their cytotoxicity toward AML cells existed, and

leukemic cells from patients were killed by  $^{111}\text{In}$ -NLS-M195 or  $^{111}\text{In}$ -M195, but the cytotoxic response among specimens was heterogeneous. **Conclusion:** NLS conjugation enhanced the nuclear uptake and cytotoxicity of  $^{111}\text{In}$ -HuM195 and  $^{111}\text{In}$ -M195 toward drug-resistant AML cell lines as well as patient specimens expressing a diversity of MDR phenotypes, including Pgp-170, BCRP1, or MRP1 transporters. Targeted Auger electron radioimmunotherapy using  $^{111}\text{In}$ -labeled anti-CD33 monoclonal antibodies modified with NLS may be able to overcome MDR and provide a means of treating chemotherapy-resistant myeloid leukemias in patients.

**Key Words:** acute myelogenous leukemia;  $^{111}\text{In}$ ; Auger electrons; CD33; multidrug resistance

**J Nucl Med 2008; 49:1546–1554**  
DOI: 10.2967/jnumed.107.047399

**A**cute myeloid leukemia (AML) is a clonal malignancy of the hematopoietic system characterized by accumulation of immature cell populations in the bone marrow or peripheral blood (1). AML is the most common type of leukemia in adults but has the lowest survival rate of all leukemias (2). Induction chemotherapy with doxorubicin, idarubicin, etoposide, and mitoxantrone induces complete remission in 65%–70% of adults with de novo AML (3–5). However, despite these intensive treatments, patients relapse because of the development of multidrug resistance (MDR) (1). Extrusion of drugs by membrane adenosine triphosphate (ATP)-dependant drug efflux pumps (ATP-binding cassette [ABC] transporters) is commonly implicated in MDR. The best characterized ABC transporter is Pgp-170, encoded by the MDR1 gene, which has been associated with poor treatment outcome in AML (6). Other drug efflux pumps include MRP1, which also contributes to MDR in AML, and MRP2-MRP9 and BCRP1, which are still under investigation and have not yet been proven to play a role in MDR in AML (1,5).

Received Sep. 15, 2007; revision accepted Mar. 19, 2008.

For correspondence or reprints contact: Raymond M. Reilly, Leslie Dan Faculty of Pharmacy, University of Toronto, 144 College St., Toronto, Ontario M5S 3M2.

E-mail: Raymond.Reilly@utoronto.ca

COPYRIGHT © 2008 by the Society of Nuclear Medicine, Inc.

Immunotherapeutic strategies could potentially circumvent MDR and yield remissions in drug-resistant AML. One promising target on myeloid leukemia cells is CD33, a 67-kDa sialoadhesion glycoprotein that is expressed in more than 90% of cases of AML (7). CD33 is also displayed by normal myeloid cells but is not present on nonhematopoietic tissues or the earliest pluripotent hematopoietic progenitor cells (7,8). This restricted distribution of CD33 has been exploited for immunotherapy of AML using the humanized anti-CD33 monoclonal antibody (mAb) HuM195 (Lintuzumab; Protein Design Labs) or the anti-CD33 mAb calicheamicin immunoconjugates ([gemtuzumab ozogamicin] Mylotarg; Wyeth). Unfortunately, HuM195 was found to have limited efficacy as a single agent and did not improve survival when combined with chemotherapy in a phase III clinical trial (9). An overall 30% response rate to gemtuzumab ozogamicin has been achieved in patients with AML (10), but calicheamicin is a substrate for Pgp-170 and is unable to kill Pgp-170-expressing leukemia cells (11).

Radioimmunotherapy (RIT) of AML using  $^{131}\text{I}$ - and  $^{90}\text{Y}$ -labeled anti-CD33 mAbs M195 and HuM195 dramatically decreased leukemic cells in the blood and bone marrow of patients, but these radioimmunoconjugates (RICs) were associated with severe myelosuppression and hepatotoxicity (12). These normal-tissue toxicities are thought to be partially due to irradiation of nontargeted cells by the long-range (2–10 mm)  $\beta$ -particles emitted by  $^{131}\text{I}$  or  $^{90}\text{Y}$  (“cross-fire” effect) (13). The Auger electron-emitting radionuclide  $^{111}\text{In}$  is an attractive alternative for labeling HuM195 for RIT of myeloid leukemias. More than 99% of the Auger electrons emitted by  $^{111}\text{In}$  have a range of less than 1  $\mu\text{m}$ , which obviates the cross-fire effect and yields high-linear-energy transfer comparable to that of highly cytotoxic  $\alpha$ -emitters (i.e., 50–100 keV/ $\mu\text{m}$ ). We previously reported that HuM195 labeled with  $^{111}\text{In}$  and modified with 13-mer peptides (CGYGPKKKRKVGG) harboring the nuclear localization sequence (NLS; underlined) of SV-40 large T-antigen was internalized and routed to the nucleus of leukemia cells, in which the Auger electrons were lethal to HL-60 myeloid leukemia cells and primary AML specimens (14).  $^{111}\text{In}$ -NLS-HuM195 was not toxic to normal tissues in BALB/c mice at doses up to 185 MBq/kg. Our hypothesis in the current study was that mitoxantrone-insensitive HL-60-MX-1 cells and primary AML specimens expressing ABC transporters would be sensitive to the cytotoxic effects of  $^{111}\text{In}$ -labeled M195 and HuM195 mAbs modified with NLS peptides. Thus, RIT using  $^{111}\text{In}$ -NLS-HuM195 could potentially offer an opportunity to treat patients with AML who have evidence of MDR at presentation or who have relapsed after induction chemotherapy due to the emergence of MDR.

## MATERIALS AND METHODS

### Human AML Cells and Primary AML Specimens

HL-60 (CCL-240) and mitoxantrone-resistant HL-60-MX-1 (CRL-2258) human leukemia cells, as well as M195 hybridoma cells (HB-10306) secreting M195 mAbs, were purchased from the

American Type Culture Collection. We determined that the concentration of mitoxantrone required to inhibit the growth of HL-60-MX-1 cells by 50% ( $\text{EC}_{50}$ ) is almost 5-fold greater than that for HL-60 cells ( $\text{EC}_{50} = 1.11$  vs. 0.26 nM, respectively). All cells were cultured at 37°C and in a 5%  $\text{CO}_2$  atmosphere in growth medium (RPMI 1640 medium; Sigma) supplemented with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 4.5 g of D-glucose per liter, 1 mM sodium pyruvate, 1% (v/v) penicillin/streptomycin (Sigma), and 10% (v/v) non-heat-inactivated fetal calf serum (FCS) (Sigma). AML specimens were collected under a protocol (no. 02-0763C) approved by the Human Subjects Ethics Review Board at the Princess Margaret Hospital, University Health Network, Toronto, Ontario. Mononuclear blood cells were isolated by Ficoll-Paque (Stem Cell Technologies) density-gradient centrifugation and cryopreserved in growth medium containing 50% (v/v) FCS and 5% (v/v) dimethyl sulfoxide (Sigma) until needed.

Special cell culture conditions using complete Methocult-4800 medium (Stem Cell Technologies) with or without supplementation with recombinant growth factors were used for the clonogenic assays of HL-60 and HL-60-MX-1 cells, as well as for primary AML specimens exposed to  $^{111}\text{In}$ -labeled anti-CD33 mAbs. Complete Methocult medium was prepared by mixing 40 mL of Methocult-4800 with 30 mL of FCS, 10 mL of a 10% (w/v) bovine serum albumin solution (Sigma), 704  $\mu\text{L}$  of 14.2 mM 2-mercaptoethanol (Sigma), 1 mL of 200 mM L-glutamine (Sigma), 1 mL of 100 $\times$  antibiotics solution (product P0781; Sigma), and 18 mL of Iscove’s modified Dulbecco’s medium (product I3390; Sigma). For primary AML specimens, complete Methocult medium was supplemented with recombinant human growth factors obtained from Peprotech. The final concentrations were 50 ng/mL for stem cell factor, 20 ng/mL for GM-CSF, 20 ng/mL for IL-3, 20 ng/mL for IL-6, 20 ng/mL for G-CSF, and 50 ng/mL for Flt-3 ligand.

### $^{111}\text{In}$ -NLS-CD33 mAbs

Humanized antihuman-CD33 HuM195 mAbs (IgG1) were provided by Protein Design Labs Inc. Mouse IgG (mIgG) was purchased from Sigma (product I5381). Anti-CD33 M195 murine mAbs were isolated from the culture medium of M195 hybridoma cells by affinity chromatography (batch method) using Protein A Sepharose (Sigma). Sodium dodecylsulfonate polyacrylamide gel electrophoresis on a 4%–20% Tris-HCl gradient gel under nonreducing conditions and Western blot analysis probed with antihuman-CD33 (eBioscience) showed a single band for M195 corresponding to an  $M_r$  of 150 kDa. Conjugation of mAbs with diethylenetriaminepentaacetic acid (DTPA) dianhydride for labeling with  $^{111}\text{In}$  was performed before NLS modification to prevent derivatization of  $\epsilon$ -amino groups of lysines on the NLS peptides—these residues are critical for nuclear importation (15). Briefly, HuM195, M195, or mIgG (100  $\mu\text{L}$  of a 5 mg/mL solution in 100 mM sodium bicarbonate, pH 8.2) were reacted with a 10-fold molar excess of DTPA dianhydride (5  $\mu\text{L}$  of a 6.7 mM solution in anhydrous dimethyl sulfoxide; Sigma) for 1 h at room temperature. Excess DTPA was removed by size-exclusion chromatography on a Sepharose G50 (Sigma) minicolumn eluted with phosphate-buffered saline (PBS), pH 7.6. Purified DTPA-conjugated antibodies were reconcentrated to 2 mg/mL on a Microcon YM-100 device (Millipore). DTPA substitution was  $1.8 \pm 0.5$  mol of DTPA per mole of antibodies ( $n = 15$ ), measured as previously reported (16).

NLS peptides were conjugated to DTPA-HuM195, M195, or mIgG mAbs (14) by introducing maleimide groups through a

reaction of 150  $\mu\text{L}$  of antibodies (2 mg/mL) with a 30-fold molar excess of sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) (Pierce) or 50  $\mu\text{L}$  of a 1.2 mM solution in PBS (pH 7.6) for 1 h at room temperature. Excess sulfo-SMCC was removed by size-exclusion chromatography on a Sepharose G50 minicolumn eluted with PBS, pH 7.0. Maleimide-derivatized DTPA antibodies (100  $\mu\text{L}$  of a 2 mg/mL solution in PBS, pH 7.0) were reacted with a 25-fold molar excess (50  $\mu\text{L}$  of a 0.67 mM solution in PBS, pH 7.0) of 13-mer peptides (CGYGPKKKRK-VGG) harboring the NLS of SV-40 large T-antigen (underlined) (Advanced Protein Technology Center, Hospital for Sick Children) for 18 h at 4°C. Excess NLS peptides were removed by size-exclusion chromatography on a Sepharose G50 minicolumn eluted with 100 mM sodium acetate, pH 6.0. NLS-conjugated antibodies were finally reconcentrated to 2 mg/mL. The level of NLS substitution was  $10 \pm 3$  NLS peptides per antibody molecule measured as previously reported (14). NLS-conjugated or unmodified DTPA-HuM195, DTPA-M195, or DTPA-mIgG was radiolabeled by incubation of 10–200  $\mu\text{g}$  of mAbs with  $^{111}\text{InCl}_3$  (MDS Nordion) for 60 min at room temperature. The radiochemical purity was  $98.2\% \pm 4.5\%$  ( $n = 75$ ), determined by instant thin-layer silica gel chromatography and size-exclusion high-performance liquid chromatography as previously reported (14), and the final specific activity (SA) was 1–8 MBq/ $\mu\text{g}$ . HuM195 or M195 mAbs were labeled with  $^{123}\text{I}$  using the IODO-GEN method (17).

### Flow Cytometry of Leukemia Cells

The immunoreactivities of HL-60, HL-60-MX-1, and AML patient specimens were assessed with mouse antibodies specific for human CD33 (anti-CD33) conjugated to fluorescein isothiocyanate or phycoerythrin-cyanine-5 (PE-cy5) and with anti-CD243-fluorescein isothiocyanate (anti-Pgp-170), anti-BCRP1-PE (anti-ABC2), and anti-MRP1-fluorescein isothiocyanate (Becton-Dickinson Pharmingen). Nonspecific binding was also assessed with isotype-matched irrelevant antibody controls.

### Radioligand Saturation Binding Assays

The CD33 binding affinities ( $K_d$ ) and maximum binding sites ( $B_{\text{max}}$ ) for  $^{111}\text{In}$ -labeled NLS-HuM195 and NLS-M195 and  $^{123}\text{I}$ -labeled HuM195 and M195 were determined in direct radioligand saturation binding assays using HL-60 and HL-60-MX-1 cells as previously reported (14). The bound radioligand concentration (mol/L) was plotted versus total radioligand concentration (mol/L), and the resulting curve was fitted to a 1-site saturation binding model using Prism software (version 4.0; GraphPad). The dissociation constant ( $K_d$ ) and  $B_{\text{max}}$  were estimated. All data points and radioligand binding experiments were performed in triplicate.

### Measurement of Nuclear Localization

The nuclear localization of  $^{111}\text{In}$ -labeled HuM195, M195, NLS-HuM195, and NLS-M195 in HL-60 and HL-60-MX-1 cells was measured by subcellular fractionation. Briefly,  $^{111}\text{In}$ -labeled mAbs (200 nM in 100  $\mu\text{L}$  of growth medium) were incubated for 3 h at 37°C with  $1 \times 10^6$  HL-60 or HL-60-MX-1 cells in microtubes. The cell suspensions were then recovered and centrifuged for 5 min at 500g, the supernatant was removed, and the cell pellet was resuspended in ice-cold PBS, pH 7.4. This procedure was repeated 3 times. Then 500  $\mu\text{L}$  of cell membrane lysis buffer (containing 1.25 mL of potassium chloride [3.73 g/50 mL], 250  $\mu\text{L}$  of magnesium chloride [10.83 g/50 mL], 333  $\mu\text{L}$  of Tris-HCl [1 g/10 mL], and 250  $\mu\text{L}$  of NP-40) was added, and the cells were incubated for 6 min at 4°C. Finally, the cell suspension was centrifuged for 5 min at

1,000g, the cytoplasmic fraction (supernatant) was removed, and the nuclear pellet was isolated and resuspended in ice-cold PBS, pH 7.4. The nuclear pellet was rinsed twice with ice-cold PBS (pH 7.4), the suspension was recentrifuged, and the supernatant was removed and combined with that previously separated. Finally, the radioactivities contained in the cytoplasmic and nuclear fractions were measured in a  $\gamma$ -counter. All data points and individual experiments were performed in triplicate.

### Cell Proliferation Assays

The antiproliferative effects of  $^{111}\text{In}$ -HuM195,  $^{111}\text{In}$ -M195,  $^{111}\text{In}$ -NLS-HuM195, or  $^{111}\text{In}$ -NLS-M195 on HL-60 and HL-60-MX-1 cells were measured by the WST-1 cell-viability assay (Roche Diagnostics). Approximately  $1 \times 10^3$  HL-60 or HL-60-MX-1 cells were suspended in 100  $\mu\text{L}$  of growth medium and seeded in replicates ( $n = 5$ ) into wells in a 96-well culture plate. Antiproliferative effects were tested at 3 different specific activities (1, 2, or 5 MBq/ $\mu\text{g}$ ). Control treatments included  $^{111}\text{In}$ -NLS-mIgG (5 MBq/ $\mu\text{g}$ ) and  $^{111}\text{In}$ -acetate as well as nonradiolabeled NLS peptides, DTPA-NLS-HuM195, or DTPA-NLS-M195. For the nonradioactive treatments, mass-equivalent concentrations identical to the highest tested for the radiolabeled mAbs (i.e., lowest specific activity) or equivalent NLS-peptide concentrations for these  $^{111}\text{In}$ -labeled antibodies were used. Increasing concentrations (0–250 kBq/well) of  $^{111}\text{In}$ -labeled mAbs in 100  $\mu\text{L}$  of growth medium were added to each well, and the cells were cultured for 7 d. WST-1 reagent (20  $\mu\text{L}$ ) was then added, and the dishes were incubated at 37°C in 5%  $\text{CO}_2$  for 2 h. The absorbance of the wells was measured at 450 and 690 nm (reference wavelength) in a microplate reader (model Elx800; Bio-Tek Instruments Inc.). Growth inhibition was defined as the ratio of the absorbance of wells containing treated cells to the absorbance of wells containing untreated cells. This ratio was plotted versus the concentration (nmol/L) of  $^{111}\text{In}$ -labeled mAbs, and the resulting curve was analyzed by nonlinear top-to-bottom regression using Prism (version 4.0). The concentrations required to inhibit cell growth by 50% ( $\text{EC}_{50}$ ) were calculated.

### Clonogenic Assays

The clonogenic survival of HL-60 or HL-60-MX cells exposed to  $^{111}\text{In}$ -M195,  $^{111}\text{In}$ -NLS-HuM195,  $^{111}\text{In}$ -NLS-M195, or  $^{111}\text{In}$ -NLS-mIgG (irrelevant control) was determined. Briefly,  $1 \times 10^6$  cells were incubated for 3 h at 37°C in Eppendorf microtubes with  $^{111}\text{In}$ -labeled mAbs (0–250 mBq/cell; specific activities of 1, 3, 6, or 8 MBq/ $\mu\text{g}$ ) contained in 60  $\mu\text{L}$  of growth medium. An additional 940  $\mu\text{L}$  of growth medium were then added, and the cell suspensions were centrifuged for 5 min at 500g. This was repeated twice. Finally, the cells were resuspended in growth medium to a concentration of  $4\text{--}5 \times 10^3$  cells/mL, and approximately 400  $\mu\text{L}$  were added to 4 mL of complete Methocult. Approximately 1 mL of this mixture was plated in triplicate into 35-mm cell culture dishes (Stem Cell Technologies). The cells were cultured at 37°C in 5%  $\text{CO}_2$  for 14 d, and the number of colonies (>100 cells) formed was counted using an inverted microscope. All experiments were performed in triplicate. Clonogenic survival was calculated by dividing the number of colonies in treated dishes by the number in untreated dishes and multiplying by 100%. Clonogenic survival versus the amount of radioactivity incubated with the cells (mBq/cell) was plotted and the resulting curve fitted to a dose-response curve,  $N = ([N_0 - \text{plateau}] \times \exp[-D/D_0] + \text{plateau})$ , where  $N$  is the number of cells surviving,  $N_0$  is the number of cells initially,  $D$  is the amount of radioactivity incubated with the cells (mBq/cell),

$D_0$  is the mean lethal amount of radioactivity (mBq/cell), and plateau is the lower asymptote of the curve (18).

The clonogenic survival of primary AML specimens exposed to  $^{111}\text{In}$ -labeled mAbs was determined by first thawing the specimens gradually over 10 min at 37°C and then rinsing the cells with Hanks' balanced salt solution (Stem Cell Technologies) containing 5% FCS. The cells were subsequently resuspended in growth medium, and  $5 \times 10^5$  cells were incubated with 0–250 mBq/cell of  $^{111}\text{In}$ -M195 or  $^{111}\text{In}$ -NLS-M195 (8 MBq/ $\mu\text{g}$ ) or  $^{111}\text{In}$ -NLS-mIgG (8 MBq/ $\mu\text{g}$ ) in 60  $\mu\text{L}$  of growth medium in microtubes for 3 h at 37°C. The cells were recovered and centrifuged at 500g. Approximately  $2 \times 10^5$  cells were resuspended in 1 mL of complete Methocult medium supplemented with growth factors and plated in triplicate into 35-mm cell culture dishes. The cells were cultured at 37°C in 5%  $\text{CO}_2$  for 14 d, and the number of colonies formed (>100 cells) was counted using an inverted microscope. The clonogenic survival was calculated, and the data were analyzed by Prism (version 4.0) as described previously.

### Statistical Analysis

Statistical significance was tested using the Mann–Whitney  $U$  test ( $P < 0.05$ ) or ANOVA with the Bonferroni adjustment ( $P < 0.05$ ).

## RESULTS

### Flow Cytometry of Leukemia Cells

CD33 was highly expressed by all 10 primary AML specimens, with 79%–99% of the cells immunopositive (Table 1). Similarly, almost 100% of HL-60 and HL-60-MX-1 cells displayed CD33 epitopes. No expression of the selected MDR transporters was found on HL-60 and HL-60-MX-1 cells. The resistance of HL-60-MX-1 cells to mitoxantrone is atypical and is due to the absence of the topoisomerase IIb isoform (19). In contrast, primary AML specimens exhibited immunoreactivity for Pgp-170 (8%–77%), BCRP1 (0.1%–14%), and MRP1 (1%–73%).

**TABLE 1**  
Expression of CD33 and MDR Transporter Epitopes on Primary AML Specimens

AML specimen	Percentage of cells immunopositive for selected epitope			
	CD33 <sup>+</sup>	Pgp-170 <sup>+</sup>	BCRP1 <sup>+</sup>	MRP1 <sup>+</sup>
1	93.5 ± 3.9	41.7 ± 1.6	0.6 ± 0.4	73.2 ± 2.2
2	97.2 ± 2.4	14.9 ± 6.3	0.1 ± 0.1	11.4 ± 4.8
3	79.2 ± 5.0	48.3 ± 3.1	10.9 ± 0.8	30.5 ± 1.1
4	86.1 ± 3.4	66.4 ± 1.9	13.8 ± 0.4	34.1 ± 2.5
5	81.8 ± 1.1	7.9 ± 0.9	0.2 ± 0.0	1.2 ± 0.2
6	87.3 ± 2.5	18.9 ± 1.1	1.2 ± 0.4	45.0 ± 3.7
7	91.5 ± 7.2	28.4 ± 1.1	8.1 ± 1.7	30.8 ± 1.8
8	98.5 ± 1.3	50.9 ± 2.6	9.2 ± 1.0	7.8 ± 1.1
9	80.3 ± 4.0	77.1 ± 5.7	3.8 ± 0.2	37.0 ± 5.3
10	99.0 ± 0.4	53.0 ± 4.9	6.9 ± 1.5	20.7 ± 1.4

Results shown are mean ± SD of percentage positive cells determined in triplicate by flow cytometry.

### Radioligand Saturation Binding Assays

NLS conjugation and  $^{111}\text{In}$ -labeling of M195 resulted in a 3-fold increased  $K_d$  for binding to HL-60 and HL-60-MX-1 cells ( $13.9 \pm 1.1$  and  $42.7 \pm 3.5$  nmol/L, respectively), compared with  $^{123}\text{I}$ -M195 ( $K_d$ ,  $4.1 \pm 0.4$  and  $14.3 \pm 1.6$  nmol/L, respectively). Modification of HuM195 with NLS and labeling with  $^{111}\text{In}$  did not significantly change the  $K_d$  for binding CD33 on HL-60 or HL-60-MX-1 cells ( $1.2 \pm 0.4$  vs.  $1.7 \pm 0.6$  nmol/L, respectively;  $P > 0.05$ ) but was 2- to 3-fold higher than that for  $^{123}\text{I}$ -HuM195 ( $0.5 \pm 0.2$  and  $0.8 \pm 0.4$  nmol/L, respectively). No significant differences in  $B_{\text{max}}$  values for binding of  $^{123}\text{I}$ -M195,  $^{123}\text{I}$ -HuM195,  $^{111}\text{In}$ -NLS-M195, or  $^{111}\text{In}$ -HuM195 to HL-60 or HL-60-MX-1 cells ( $3\text{--}4 \times 10^4$  CD33 epitopes per cell) were exhibited.

### Measurement of Nuclear Localization

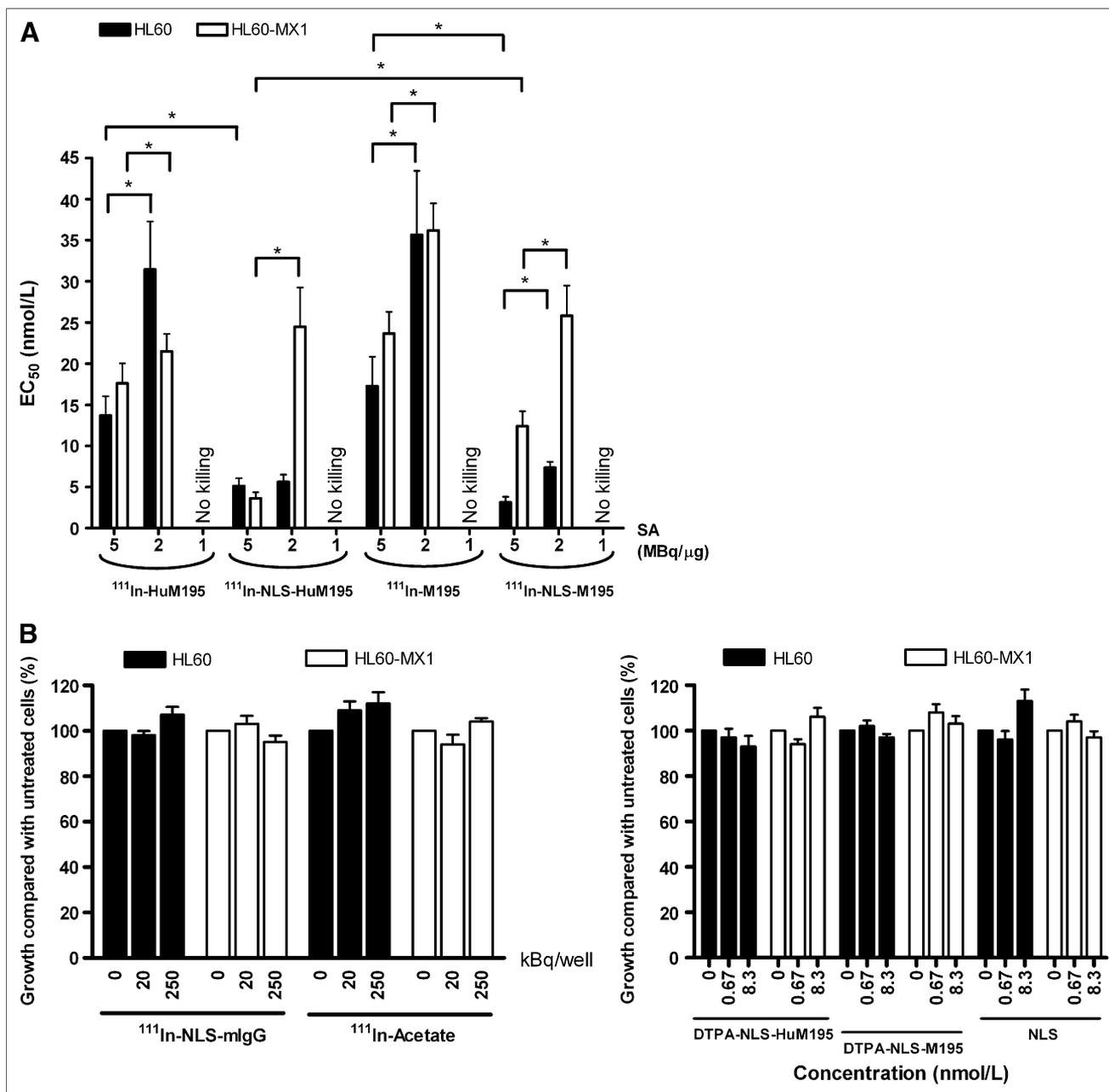
The percentage of radioactivity imported into the nucleus for  $^{111}\text{In}$ -M195 and  $^{111}\text{In}$ -NLS-M195 was  $4.4 \pm 0.1$  versus  $22.4 \pm 6.3$  ( $P < 0.05$ ) in HL-60 cells and  $5.0 \pm 1.4$  versus  $18.5 \pm 4.4$  ( $P < 0.05$ ) in HL-60-MX-1 cells. The percentage of nuclear radioactivity for  $^{111}\text{In}$ -HuM195 and  $^{111}\text{In}$ -NLS-HuM195 was  $5.8 \pm 0.6$  versus  $24.1 \pm 3.2$  ( $P < 0.05$ ) in HL-60 cells and  $5.1 \pm 0.8$  versus  $19.7 \pm 3.4$  ( $P < 0.05$ ) in HL-60-MX-1 cells. These results were consistent with those previously reported for HL-60 cells incubated with  $^{111}\text{In}$ -HuM195 modified with 4–8 NLS peptides (14). No significant differences in the proportion of nuclear localization of radioactivity between HL-60 and HL-60-MX-1 cells for  $^{111}\text{In}$ -NLS-M195 or  $^{111}\text{In}$ -NLS-HuM195 were demonstrated.

### Cell Proliferation Assays

Growth inhibition of HL-60 and HL-60-MX-1 cells by the  $^{111}\text{In}$ -labeled mAbs with or without NLS modification was greater for an SA of 5 MBq/ $\mu\text{g}$  than for that of 2 MBq/ $\mu\text{g}$  (Fig. 1A). No growth inhibition was found at an SA of 1 MBq/ $\mu\text{g}$ . At the highest SA tested, NLS incorporation resulted in significantly lower  $\text{EC}_{50}$  values, compared with unmodified  $^{111}\text{In}$ -HuM195 and  $^{111}\text{In}$ -M195 mAbs. No significant differences between  $^{111}\text{In}$ -NLS-HuM195 and  $^{111}\text{In}$ -NLS-M195 in the ability to inhibit the growth of HL-60 cells were exhibited. HL-60-MX-1 cells, however, were as sensitive as HL-60 cells to  $^{111}\text{In}$ -NLS-HuM195 but were significantly less growth-inhibited by the murine antibody form  $^{111}\text{In}$ -NLS-M195. At the highest concentration (1.7 nmol/L) and SA tested (5 MBq/ $\mu\text{g}$ ), the growth of HL-60 and HL-60-MX-1 cells were reduced to  $3.2\% \pm 1.1\%$  and  $5.1\% \pm 2.4\%$ , respectively, by  $^{111}\text{In}$ -NLS-HuM195 and to  $1.8\% \pm 1.4\%$  and  $2.7\% \pm 1.6\%$ , respectively, by  $^{111}\text{In}$ -NLS-M195. Neither cell line was affected by exposure to  $^{111}\text{In}$ -NLS-mIgG or  $^{111}\text{In}$ -acetate or to nonradiolabeled NLS peptides, DTPA-NLS-HuM195, and DTPA-NLS-M195 (Fig. 1B).

### Clonogenic Assays

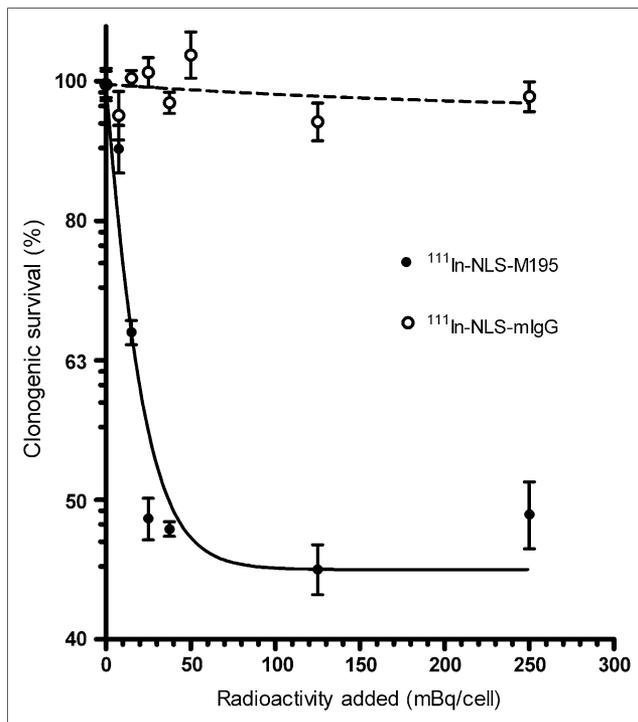
A representative clonogenic survival curve for HL-60-MX-1 cells treated with  $^{111}\text{In}$ -NLS-M195 is shown in



**FIGURE 1.** (A) Growth inhibition of HL-60 and HL-60-MX-1 cells by <sup>111</sup>In-HuM195, <sup>111</sup>In-NLS-HuM195, <sup>111</sup>In-M195, or <sup>111</sup>In-NLS-M195 at 3 different specific activities measured using WST-1 cell viability assay. Results are expressed as mean  $\pm$  SD of EC<sub>50</sub> values ( $n = 3$ ). Significant differences ( $P < 0.05$ ) are indicated by asterisks. (B) Effect of <sup>111</sup>In-NLS-mIgG, <sup>111</sup>In-acetate, DTPA-NLS-M195, or NLS peptides at increasing radioactivity or mass concentrations on growth of HL-60 and HL-60-MX-1 cells. Results are expressed as mean  $\pm$  SD of percentage cell growth ( $n = 3$ ) relative to untreated cells.

Figure 2, and the results of these assays for both cell lines at an SA of 3–8 MBq/μg are summarized in Table 2. No effects on the clonogenic survival of HL-60 or HL-60-MX-1 cells were observed with <sup>111</sup>In-labeled mAbs at 1 MBq/μg, and these results are not shown. The survival of HL-60 or HL-60-MX-1 cells was reduced by more than 60% as indicated by the asymptotic values (percentage of cells surviving at the highest concentration of radioactivity added). Moreover, these asymptotic values were lower for <sup>111</sup>In-NLS-M195 and <sup>111</sup>In-NLS-HuM195 than those for <sup>111</sup>In-

M195 without NLS peptides and were systematically decreased with increasing SA. The D<sub>0</sub> values represent the mean lethal concentration (mBq/cell) added to the cells. At the highest SA tested (8 MBq/μg), D<sub>0</sub> values were 1.5- to 3-fold significantly greater for HL-60-MX-1 than for HL-60 cells. The D<sub>0</sub> values were 2-fold significantly lower for <sup>111</sup>In-NLS-HuM195 than for <sup>111</sup>In-NLS-M195. D<sub>0</sub> and asymptotic values were not calculated for <sup>111</sup>In-NLS-mIgG, because it had no effect on the clonogenic survival of either cell line.



**FIGURE 2.** Representative clonogenic survival curve for HL-60-MX-1 cells after treatment with  $^{111}\text{In}$ -NLS-M195 or  $^{111}\text{In}$ -NLS-mIgG at SA of 8 MBq/ $\mu\text{g}$ . Results are expressed as mean  $\pm$  SD ( $n = 3$ ) of percentage cell survival relative to untreated cells.

The results of clonogenic assays of 10 primary AML specimens exposed to  $^{111}\text{In}$ -NLS-M195 or  $^{111}\text{In}$ -M195 are shown in Table 3.  $^{111}\text{In}$ -NLS-M195 and  $^{111}\text{In}$ -M195 decreased the clonogenic survival (lower asymptote) to 23%–49% and 35%–61%, respectively. Comparisons for individual

specimens revealed that no significant differences in the  $D_0$  values for  $^{111}\text{In}$ -NLS-M195 and  $^{111}\text{In}$ -M195 existed, but the asymptotic values were significantly lower in all cases for  $^{111}\text{In}$ -NLS-M195.

## DISCUSSION

In this study, we showed that the Auger electron emissions from the  $^{111}\text{In}$ -labeled anti-CD33 mAbs HuM195 and M195 modified with NLS peptides significantly diminished the survival in vitro of mitoxantrone-resistant HL-60-MX-1 myeloid leukemia cells and primary AML specimens expressing ABC MDR transporters. This finding creates new opportunities to circumvent MDR in AML and possibly achieve remissions in patients refractory to chemotherapy, which is a major obstacle in the effective clinical management of this disease (20). Other approaches to overcoming drug resistance in AML have focused on the use of selective MDR1 inhibitors such as PSC-883 or quinine, but these have proven relatively ineffective in clinical trials (21,22). Newer inhibitors are under development that may be more effective, but they may increase toxicity from chemotherapy, because of the presence of MDR1 transporters in drug-eliminating organs (8). Auger electron therapy using  $^{125}\text{I}$ -labeled 4'-thio-2'-deoxyuridine, on the other hand, has proven successful in treating chemoresistant and radioresistant HL-60 cells (23). Targeted Auger electron RIT with  $^{111}\text{In}$ -NLS-HuM195 or  $^{111}\text{In}$ -NLS-M195 is not expected to be severely limited by normal-tissue toxicity, because CD33-mediated internalization and NLS-promoted nuclear importation (24) are required for manifestation of the cytotoxic effects of the Auger electrons (14,25).

Nuclear radioactivity for NLS-modified  $^{111}\text{In}$ -labeled M195 and HuM195 (18%–24%) was 4- to 6-fold greater

**TABLE 2**

$D_0$  and Lower Asymptote for Clonogenic Survival of HL-60 and Mitoxantrone-Resistant HL-60-MX-1 Cells Exposed to Radioconjugates at Different Specific Activities

Radioimmunoconjugate and specific activity (MBq/ $\mu\text{g}$ )	HL-60		HL-60-MX-1	
	$D_0$ (mBq/cell)	Lower asymptote (% cells surviving)	$D_0$ (mBq/cell)	Lower asymptote (% cells surviving)
$^{111}\text{In}$ -M195				
3	17.1 $\pm$ 3.6	53 $\pm$ 4	ND	ND
6	19.5 $\pm$ 2.1	53 $\pm$ 2	20.2 $\pm$ 2.6	55 $\pm$ 2
8	9.3 $\pm$ 1.3*	61 $\pm$ 2*	16.4 $\pm$ 2.4*	55 $\pm$ 2*
$^{111}\text{In}$ -NLS-M195				
3	25.3 $\pm$ 4.0	56 $\pm$ 3	24.3 $\pm$ 2.6	55 $\pm$ 1
6	16.7 $\pm$ 2.3	50 $\pm$ 5	13.5 $\pm$ 1.1	34 $\pm$ 1
8	9.8 $\pm$ 0.8*	43 $\pm$ 1*	15.8 $\pm$ 0.5*	38 $\pm$ 1*
$^{111}\text{In}$ -NLS-HuM195				
3	23.8 $\pm$ 4.9	67 $\pm$ 4	ND	ND
6	22.5 $\pm$ 4.3	60 $\pm$ 3	16.4 $\pm$ 3.0	54 $\pm$ 4
8	5.6 $\pm$ 0.6*	42 $\pm$ 1*	16.1 $\pm$ 1.9*	44 $\pm$ 4*

\*Significantly different values ( $P < 0.05$ ).

Values shown are mean  $\pm$  SD of triplicate determinations. No effects on clonogenic survival of HL-60 or HL-60-MX-1 cells were observed for  $^{111}\text{In}$ -labeled mAbs at 1 MBq/ $\mu\text{g}$  (not shown). ND = not determined.

TABLE 3

D<sub>0</sub> and Lower Asymptote for Clonogenic Survival of Primary AML Specimens Exposed to <sup>111</sup>In-M195 or <sup>111</sup>In-NLS-M195 at Specific Activity of 8 MBq/μg

AML specimen	<sup>111</sup> In-M195		<sup>111</sup> In-NLS-M195	
	D <sub>0</sub> (mBq/cell)	Lower asymptote (% cells surviving)*	D <sub>0</sub> (mBq/cell)	Lower asymptote (% cells surviving)*
1	29.4 ± 3.8	52 ± 4	34.1 ± 4.2	33 ± 3
2	16.1 ± 0.9	41 ± 2	13.6 ± 1.4	23 ± 2
3	5.1 ± 0.5	35 ± 3	4.3 ± 0.1	26 ± 1
4	12.2 ± 1.8	49 ± 3	9.4 ± 1.2	31 ± 2
5	6.1 ± 0.3	51 ± 1	5.6 ± 0.7	35 ± 3
6	5.5 ± 0.3	54 ± 2	5.8 ± 0.5	43 ± 4
7	10.4 ± 0.9	57 ± 2	9.1 ± 1.4	46 ± 4
8	4.5 ± 0.6	58 ± 1	4.7 ± 0.7	47 ± 2
9	6.6 ± 0.7	54 ± 3	6.4 ± 0.3	43 ± 1
10	17.3 ± 0.4	61 ± 5	16.7 ± 1.1	49 ± 1

\*Significantly different values ( $P < 0.05$ ).

Values shown are mean ± SD of triplicate determinations.

than for the corresponding RICs without NLS modification. These results confirm those previously reported for <sup>111</sup>In-NLS-HuM195 in HL-60 cells (14) but now extend these findings to drug-resistant HL-60-MX-1 cells. Nuclear localization is critical for exploiting the Auger electrons emitted by <sup>111</sup>In for targeted radiotherapy of AML, because cellular radiation dosimetry models have revealed that the radiation-absorbed dose to the nucleus is amplified more than 34 times from <sup>111</sup>In deposited in the nucleus, compared with on the cell surface, and is 17 times greater for nuclear than for cytoplasmic <sup>111</sup>In (25). The efficiencies of nuclear localization of <sup>111</sup>In-NLS-M195 or <sup>111</sup>In-NLS-HuM195 were equivalent for HL-60 and HL-60-MX-1 cells and were not affected by expression of the drug-resistance phenotype. Assuming that a single HL-60 or HL-60-MX-1 cell was targeted to CD33 saturation ( $3 \times 10^4$  receptors/cell) with <sup>111</sup>In-NLS-HuM195 or <sup>111</sup>In-NLS-M195 at an SA of 8 MBq/μg, that 10% of the radioactivity is localized to the cell surface, that 70% is deposited into the cytoplasm, and that 20% is deposited into the nucleus with elimination only by radioactive decay, we estimate that the maximum radiation-absorbed dose to the nucleus would be about 3,067 cGy using the cellular dosimetry model of Goddu et al. (25).

Growth inhibition and decreased clonogenic survival of HL-60 and HL-60-MX-1 cells were strongly dependent on the SA of <sup>111</sup>In-NLS-HuM195 and <sup>111</sup>In-NLS-M195, with a minimum of 2–3 MBq/μg required for cytotoxicity (Fig. 1; Table 2). At the lowest SA (1 MBq/μg), only 1 antibody molecule in 10 is complexed to <sup>111</sup>In, and because there are  $3\text{--}4 \times 10^4$  CD33 binding sites per cell, a maximum of  $3\text{--}4 \times 10^3$  <sup>111</sup>In atoms can be bound to the cell surface. Because only 20% of cell-bound and internalized <sup>111</sup>In-NLS-HuM195 and <sup>111</sup>In-NLS-M195 molecules will be transported into the nucleus, this further diminishes the effectiveness

of these low SA RICs. However, at the higher SA examined (3, 6, or 8 MBq/μg), substantially more <sup>111</sup>In atoms would reach the nucleus, in which they can cause lethal DNA damage. This premise is in agreement with the cytotoxic effects of HuM195 labeled with the α-emitter <sup>213</sup>Bi reported by Nikula et al. (26), who similarly found a strong effect of SA on the clonogenic survival of HL-60 cells. In our study, at the highest SA tested (8 MBq/μg), <sup>111</sup>In-NLS-HuM195 exhibited a 2-fold significantly lower D<sub>0</sub> value for HL-60 cells than did <sup>111</sup>In-NLS-M195 ( $5.6 \pm 0.6$  vs.  $9.8 \pm 0.8$  mBq/cell, respectively;  $P < 0.05$ ). This effect was not observed for lower SA RICs or for HL-60-MX-1 cells. In fact, the D<sub>0</sub>-values for HL-60-MX-1 cells were 1.5- to 3-fold significantly higher than those for HL-60 cells exposed to <sup>111</sup>In-NLS-HuM195, <sup>111</sup>In-NLS-M195, or <sup>111</sup>In-M195 at an SA of 8 MBq/μg, suggesting that HL-60-MX-1 cells are modestly less sensitive to the cytotoxic effects of the Auger electrons. The reason for the apparently greater potency of high-SA <sup>111</sup>In-NLS-HuM195 for HL-60 cells, compared with the potency of <sup>111</sup>In-NLS-M195, is not known but may be related to its 11-fold higher binding affinity ( $K_d$ ,  $1.2 \pm 0.4$  vs.  $13.9 \pm 1.1$  nmol/L, respectively). However, the partial resistance of HL-60-MX-1 cells, compared with that of HL-60 cells, to <sup>111</sup>In-NLS-HuM195 cannot be explained by differences in binding affinity because the  $K_d$  value for HL-60-MX-1 cells ( $1.7 \pm 0.6$  nmol/L) was not significantly different from that for HL-60 cells. Partial resistance cannot be explained by differences in nuclear-importation efficiency either, because these were equivalent for both cell lines. It is possible both that DNA repair may account for the effects of increasing SA on the cytotoxic potency of the RICs and that variability in repair capacity or repair rates may account for the differences in responsiveness between the 2 leukemia cell lines (27).

Although the cell proliferation assays demonstrated that a high proportion (95%–98%) of the growth of HL-60 or HL-60-MX-1 cells was inhibited by exposure to high concentrations (1.7 nmol/L) and specific activities (5 MBq/ $\mu$ g) of  $^{111}\text{In}$ -NLS-HuM195 or  $^{111}\text{In}$ -NLS-M195 (Fig. 1A), the conditions under which the clonogenic assays were conducted revealed a lower asymptotic value of 25%–40% survival for these cells or for primary AML specimens (Tables 2 and 3). These asymptotic values were diminished for  $^{111}\text{In}$ -labeled RICs at increasing SAs and with NLS-peptide conjugation. A short incubation period (3 h) was used to deliver the RICs to leukemic cells in the clonogenic assays, whereas in the cell proliferation experiments, these cells were exposed continuously to the RICs for 7 d. Recycling and repopulation of CD33 due to de novo synthesis may have allowed much more  $^{111}\text{In}$ -NLS-HuM195 or  $^{111}\text{In}$ -NLS-M195 to bind, internalize, and be transported to the nucleus in these cell proliferation assays, thus enhancing the cytotoxic effect. Such receptor recycling providing high cytotoxicity from Auger electrons has been previously reported for lymphoma cells exposed to  $^{111}\text{In}$ -anti-CD74 mAbs (28).

A wide range of Pgp-170 and MRP1 expression in AML specimens was demonstrated, with some specimens exhibiting a high proportion of immunopositive cells (Table 1). In contrast, the prevalence of BCRP1 expression was much lower. No significant correlations between the cytotoxic response of AML specimens to  $^{111}\text{In}$ -NLS-M195 measured by the  $D_0$  and plateau values (Table 3) and the expression of any of these drug-resistance transporters ( $r^2$  values,  $-0.322$ – $0.250$ ;  $P > 0.05$ ) were demonstrated. These results confirmed the ability of  $^{111}\text{In}$ -NLS-M195 to decrease the survival of mitoxantrone-resistant HL-60-MX-1 cells and further demonstrated that this effect extends to primary AML specimens with a diversity of MDR phenotypes. Taken together, these results are promising for the treatment of chemotherapy-resistant AML in patients using  $^{111}\text{In}$ -NLS-conjugated anti-CD33 mAbs. Humanized analogs such as  $^{111}\text{In}$ -NLS-HuM195 would be preferred, because of the well-recognized development of human antimouse antibodies in patients who are administered murine mAbs (29).

## CONCLUSION

$^{111}\text{In}$ -labeled anti-CD33 mAbs HuM195 and M195 modified with NLS were routed efficiently and equivalently to the nucleus of wild-type HL-60 myeloid leukemia cells and the mitoxantrone-resistant subclone, HL-60-MX-1, after CD33-mediated internalization. Nuclear delivery enhanced the cytotoxicity of the Auger electrons emitted by  $^{111}\text{In}$ -NLS-HuM195 and  $^{111}\text{In}$ -NLS-M195. Decreased cell growth and clonogenic survival were achieved for both HL-60 and HL-60-MX-1 cells and for primary AML specimens from patients expressing MDR phenotypes. Targeted Auger electron RIT using  $^{111}\text{In}$ -labeled anti-CD33 mAbs modified with NLS may be able to overcome MDR and provide a means of treating chemotherapy-resistant myeloid leukemias in patients.

## ACKNOWLEDGMENTS

We gratefully acknowledge the scientific advice and technical assistance provided by Drs. Liqing Jin and John Dick (University Health Network). This research was supported by grant 166591 from the Canadian Institutes of Health Research (CIHR) to Raymond M. Reilly and by a Wyeth-CIHR fellowship (DFW-77882) to Veerle Kersemans. Parts of this study were presented at the 54th Annual Meeting of the Society of Nuclear Medicine, Washington, DC, June 2–6, 2007, and at the Annual Congress of the European Association of Nuclear Medicine, Athens, Greece, October 1–4, 2006.

## REFERENCES

- van der Kolk DM, de Vries EG, Muller M, Vellenga E. The role of drug efflux pumps in acute myeloid leukemia. *Leuk Lymphoma*. 2002;43:685–701.
- Deschler B, Lubbert M. Acute myeloid leukemia: epidemiology and etiology. *Cancer*. 2006;107:2099–2107.
- Estey E, Dohner H. Acute myeloid leukaemia. *Lancet*. 2006;368:1894–1907.
- Lowenberg B, Suci S, Archimbaud E, et al. Mitoxantrone versus daunorubicin in induction-consolidation chemotherapy: the value of low-dose cytarabine for maintenance of remission, and an assessment of prognostic factors in acute myeloid leukemia in the elderly—final report. European Organization for the Research and Treatment of Cancer and the Dutch-Belgian Hemato-Oncology Cooperative Hovon Group. *J Clin Oncol*. 1998;16:872–881.
- Tazzari PL, Cappellini A, Ricci F, et al. Multidrug resistance-associated protein 1 expression is under the control of the phosphoinositide 3 kinase/Akt signal transduction network in human acute myelogenous leukemia blasts. *Leukemia*. 2007;21:427–438.
- Borg AG, Burgess R, Green LM, Scheper RJ, Yin JA. Overexpression of lung-resistance protein and increased P-glycoprotein function in acute myeloid leukaemia cells predict a poor response to chemotherapy and reduced patient survival. *Br J Haematol*. 1998;103:1083–1091.
- Ravandi F, Kantarjian H, Giles F, Cortes J. New agents in acute myeloid leukemia and other myeloid disorders. *Cancer*. 2004;100:441–454.
- Linenberger ML. CD33-directed therapy with gemtuzumab ozogamicin in acute myeloid leukemia: progress in understanding cytotoxicity and potential mechanisms of drug resistance. *Leukemia*. 2005;19:176–182.
- Feldman EJ, Brandwein J, Stone R, et al. Phase III randomized multicenter study of a humanized anti-CD33 monoclonal antibody, lintuzumab, in combination with chemotherapy, versus chemotherapy alone in patients with refractory or first-relapsed acute myeloid leukemia. *J Clin Oncol*. 2005;23:4110–4116.
- Sievers EL, Larson RA, Stadtmauer EA, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol*. 2001;19:3244–3254.
- Linenberger ML, Hong T, Flowers D, et al. Multidrug-resistance phenotype and clinical responses to gemtuzumab ozogamicin. *Blood*. 2001;98:988–994.
- Burke JM, Jurcic JG, Scheinberg DA. Radioimmunotherapy for acute leukemia. *Cancer Control*. 2002;9:106–113.
- Reilly RM. Biopharmaceuticals as targeting vehicles for in situ radiotherapy of malignancies. In: Knaeblein J, ed. *Modern Biopharmaceuticals*. Weinheim, Germany: Wiley-VCH; 2005:497–535.
- Chen P, Wang J, Hope K, et al. Nuclear localizing sequences promote nuclear translocation and enhance the radiotoxicity of the anti-CD33 monoclonal antibody HuM195 labeled with  $^{111}\text{In}$  in human myeloid leukemia cells. *J Nucl Med*. 2006;47:827–836.
- Leung SW, Harreman MT, Hodel MR, Hodel AE, Corbett AH. Dissection of the karyopherin alpha nuclear localization signal (NLS)-binding groove: functional requirements for NLS binding. *J Biol Chem*. 2003;278:41947–41953.
- Reilly R, Marks A, Law J, Lee N, Houle S. In vitro stability of EDTA and DTPA immunoconjugates of monoclonal antibody 2G3 labeled with indium-111. *Appl Radiat Isot*. 1992;43:961–967.
- Fraker PJ, Speck JC Jr. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3 $\alpha$ ,6 $\alpha$ -diphenylglycoluril. *Biochem Biophys Res Commun*. 1978;80:849–857.
- Kowalsky RJ, Falen S. Radiation biology. In: Kowalsky RJ, Falen S, eds. *Radiopharmaceuticals in Nuclear Pharmacy and Nuclear Medicine*. Washington, DC: American Pharmaceutical Association; 2004:135–139.

19. Boland MP, Fitzgerald KA, O'Neill LA. Topoisomerase II is required for mitoxantrone to signal nuclear factor kappa B activation in HL60 cells. *J Biol Chem*. 2000;275:25231–25238.
20. Milas L, Raju U, Liao Z, Ajani J. Targeting molecular determinants of tumor chemo-radioresistance. *Semin Oncol*. 2005;32(suppl 9):S78–S81.
21. Solary E, Drenou B, Campos L, et al. Quinine as a multidrug resistance inhibitor: a phase 3 multicentric randomized study in adult de novo acute myelogenous leukemia. *Blood*. 2003;102:1202–1210.
22. van der Holt B, Lowenberg B, Burnett AK, et al. The value of the MDR1 reversal agent PSC-833 in addition to daunorubicin and cytarabine in the treatment of elderly patients with previously untreated acute myeloid leukemia (AML), in relation to MDR1 status at diagnosis. *Blood*. 2005;106:2646–2654.
23. Reske SN, Deisenhofer S, Glatting G, et al.  $^{123}\text{I}$ -ITdU-mediated nanoirradiation of DNA efficiently induces cell kill in HL60 leukemia cells and in doxorubicin-,  $\beta$ -, or  $\gamma$ -radiation-resistant cell lines. *J Nucl Med*. 2007;48:1000–1007.
24. Alvisi G, Poon IK, Jans DA. Tumor-specific nuclear targeting: promises for anti-cancer therapy? *Drug Resist Updat*. 2006;9:40–50.
25. Goddu SM, Howell RW, Rao DV. Cellular dosimetry: absorbed fractions for monoenergetic electron and alpha particle sources and S-values for radionuclides uniformly distributed in different cell compartments. *J Nucl Med*. 1994;35:303–316.
26. Nikula TK, McDevitt MR, Finn RD, et al. Alpha-emitting bismuth cyclohexylbenzyl DTPA constructs of recombinant humanized anti-CD33 antibodies: pharmacokinetics, bioactivity, toxicity and chemistry. *J Nucl Med*. 1999;40:166–176.
27. McLornan DP, McMullin MF, Johnston P, Longley DB. Molecular mechanisms of drug resistance in acute myeloid leukaemia. *Expert Opin Drug Metab Toxicol*. 2007;3:363–377.
28. 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;81:985–992.
29. Reilly RM, Sandhu J, Alvarez-Diez T, Gallinger S, Kirsh J, Stern HS. Problems of delivery of monoclonal antibodies: pharmacokinetic and pharmaceutical solutions. *Clin Pharmacokinet*. 1995;28:126–142.



The Journal of  
NUCLEAR MEDICINE

## Drug-Resistant AML Cells and Primary AML Specimens Are Killed by <sup>111</sup>In-Anti-CD33 Monoclonal Antibodies Modified with Nuclear Localizing Peptide Sequences

Veerle Kersemans, Bart Cornelissen, Mark D. Minden, Joseph Brandwein and Raymond M. Reilly

*J Nucl Med.* 2008;49:1546-1554.

Published online: August 14, 2008.

Doi: 10.2967/jnumed.107.047399

---

This article and updated information are available at:

<http://jnm.snmjournals.org/content/49/9/1546>

---

Information about reproducing figures, tables, or other portions of this article can be found online at:

<http://jnm.snmjournals.org/site/misc/permission.xhtml>

Information about subscriptions to JNM can be found at:

<http://jnm.snmjournals.org/site/subscriptions/online.xhtml>

*The Journal of Nuclear Medicine* is published monthly.  
SNMMI | Society of Nuclear Medicine and Molecular Imaging  
1850 Samuel Morse Drive, Reston, VA 20190.  
(Print ISSN: 0161-5505, Online ISSN: 2159-662X)

© Copyright 2008 SNMMI; all rights reserved.

The logo for the Society of Nuclear Medicine and Molecular Imaging (SNMMI) features the letters 'S', 'N', 'M', and 'I' in a stylized, overlapping arrangement. The 'S' and 'N' are in the top row, and the 'M' and 'I' are in the bottom row. The letters are white with a red outline, set against a red background.  
SOCIETY OF  
NUCLEAR MEDICINE  
AND MOLECULAR IMAGING