In Vitro Evaluation of Targeted Antisense ^{177}Lu Radiotherapy

ETHAN R. BALKIN[1,2], FANG JIA[2,3], WILLIAM H. MILLER[4,5], and MICHAEL R. LEWIS[1,2,3,4]

[1]Area of Pathobiology, [3]Department of Veterinary Medicine and Surgery, and [4]Nuclear Science and Engineering Institute, University of Missouri-Columbia, MO, U.S.A.; [2]Harry S. Truman Memorial Veterans' Hospital, Columbia, MO, U.S.A.; [5]University of Missouri Research Reactor Center, Columbia, MO, U.S.A.

Abstract. *Background: The BCL2 proto-oncogene in non-Hodgkin's lymphoma is a dominant inhibitor of apoptosis. The goal of this work was to develop a ^{177}Lu-labeled anti-BCL2-peptide nucleic acid (PNA) conjugate designed for dual modality NHL therapy, i.e., simultaneous down-regulation of BCL2-mediated resistance to apoptosis and delivery of cytotoxic internally emitted radiation. Materials and Methods: The effect of 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetra-acetic acid (DOTA)-anti-BCL2-Tyr^3-octreotate was evaluated by uptake, efflux, proliferation, and viability assays, using Mec-1 lymphoma cells. In vitro dosimetry was modeled with a Monte Carlo projection. Results: Cellular efflux indicated moderate retention of radioactivity in the Mec-1 cells. Viability studies using the ^{177}Lu-labeled PNA conjugate indicated a mass-dose dependence and strongly additive statistical effect in reducing cellular viability. Conclusion: These studies demonstrate the ability of a BCL2 antisense PNA conjugate to specifically target, be retained in, and reduce cellular viability in Mec-1 NHL cells. The results also hold promise for the development of a therapeutic radiopharmaceutical with potential dual modality function.*

The protein product of the B-cell lymphoma/leukemia-2 (*BCL2*) proto-oncogene is a dominant inhibitor of apoptosis. In aggressive non-Hodgkin's lymphoma (NHL), large cohort studies have shown that overexpression of the *BCL2* gene correlates strongly with resistance to radiation and chemotherapy, increased survival of cancer cells, high relapse rate, and poor disease-free and overall survival (1-5). Thus, patients whose tumors are found to overexpress *BCL2* might respond better to alternative treatments such as targeted immunotherapy, radioimmunotherapy, or antisense therapy, all of which act through mechanisms that down-regulate *BCL2*. Human NHL also expresses type 2 somatostatin receptors (SSTR2) in approximately 87% of cases (6). Previous work indicated that ^{177}Lu-1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetra-acetic acid (DOTA)-Tyr^3-octreotate, a somatostatin-like radiopharmaceutical, provides effective, selectively targeted radiotherapy in human SSTR-expressing tumors (7-12). Furthermore, this peptide is an attractive vehicle for delivery of intracellular tumor-targeting agents, such as those designed to act against *BCL2* (13-16).

Lutetium-177 is a very promising therapeutic radionuclide. A readily available reactor-produced lanthanide, it has several very favorable physical properties suitable for targeted radiotherapy. With a 6.71 day half-life, the principal mode of decay is β^- emission with a maximum energy of 497 keV (90%) and two low abundance γ emissions of approximately 113 (6%) and 208 (11%) keV respectively, suitable for tracking radiopharmaceuticals and for radiation dosimetry *in vivo* (17, 18). Additionally the coordination chemistry of the radiometal lends itself to forming stable complexes with DOTA quite readily (18).

The present work addresses the hypothesis that combined ^{177}Lu and *BCL2* antisense therapy act synergistically or additively with respect to cell proliferation and viability in an *in vitro* model of B-cell lymphoma. *In vitro* uptake, efflux, proliferation, and viability assays were designed to assess the targeting of a ^{177}Lu-labeled BCL2 antisense peptide nucleic acid (PNA)–peptide conjugate (Figure 1), against human NHL cells in suspension culture. Previous work described an ^{111}In-labeled BCL2 antisense PNA–peptide conjugate, using Tyr3-octreotate for SSTR2 receptor-mediated delivery for the molecular imaging of *BCL2* in cell and mouse models of NHL (19). The results of those experiments demonstrated specific tumor targeting, imaging, and reduction of *BCL2* protein synthesis by 51% in

Correspondence to: Michael R. Lewis, Ph.D., Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, 900 East Campus Drive, University of Missouri-Columbia, Columbia, MO 65211, U.S.A. Tel: +1 573 8146000 ext. 53703, Fax: +1 573 8146551, e-mail: LewisMic@missouri.edu

Key Words: Non-Hodgkin's lymphoma, BCL2, lutetium-177, somatostatin receptor.

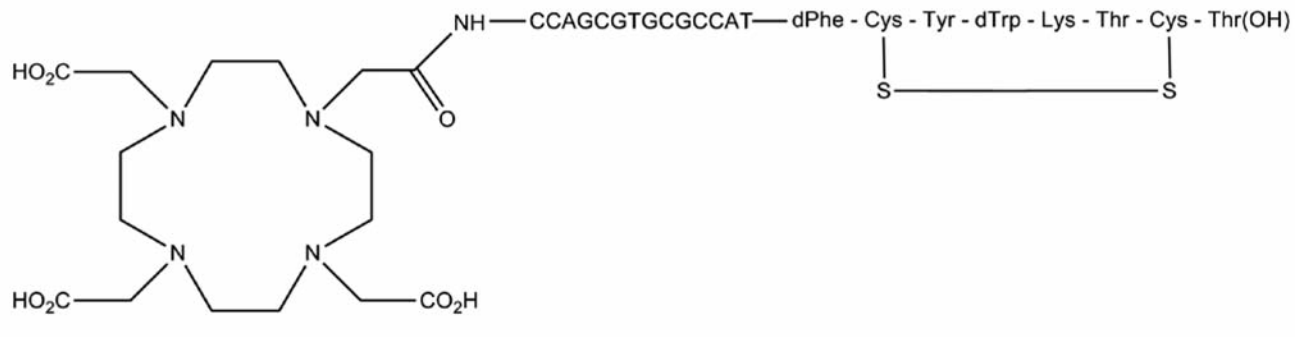

Figure 1. *Structure of DOTA-anti-BCL2-Tyr^3-octreotate.*

NHL cells, establishing precedence for this work. The human small lymphocytic lymphoma (SLL) cell line Mec-1, which expresses somatostatin receptors for delivery and *BCL2* mRNA for targeting, served as the tumor cell model for the present studies. This article reports the development of a cytotoxic ^{177}Lu-labeled antisense agent capable of specifically targeting *BCL2*.

Materials and Methods

PNA–peptide synthesis. DOTA-Tyr^3-octreotate was synthesized on an Advanced ChemTech (Louisville, KY, USA) 396-V multiple synthesizer, using standard Fmoc chemistry. Peptidyl-resins were transferred to a manual reaction vessel where PNA synthesis was performed using modifications of methods described previously by Gallazzi *et al*. and Lewis *et al*. (20, 21). DOTA was coupled at the end of the synthesis using DOTA tris-*tert*-butyl ester. The conjugates were deprotected and detached from the resin by treatment for 4 h with trifluoroacetic acid (TFA) (87.5%) and 2.5% of each of the following scavengers: *m*-cresol, H_2O, phenol, thioanisole, 1,2-ethanedithiol, and triisopropylsilane. Precipitation and multiple washing with diethyl ether yielded the crude conjugates, which were analyzed by liquid chromatography–electrospray ionization mass spectrometry (LC-ESI-MS). Disulfide cyclization was accomplished by dimethyl sulfoxide (DMSO) oxidation of crude conjugates. Each compound was purified by semi-preparative reversed phase HPLC (RP-HPLC), using a stepwise gradient of 0-20% solvent B (solvent A: 0.1% TFA/H_2O; solvent B: 0.1% TFA/CH_3CN) in 10 min followed by 20-40% solvent B in 60 min. Fractions corresponding to the desired products were pooled, lyophilized and analyzed for purity by LC-ESI-MS. Typical purity of the PNA- peptide conjugate was found to be between 95 and 98%.

^{177}Lu-labeling of conjugates. The representative conditions for labeling the DOTA–PNA–peptide conjugate with ^{177}Lu given here were based on previously published methods (19, 21). To 185 MBq of $^{177}LuCl_3$ in 550-600 μl of 0.2 M ammonium acetate, pH 5.0, containing approximately 1 mg/ml of gentisic acid and 0.1% Tween-80, was added 100 μg of DOTA–PNA–peptide in 120-200 μl of H_2O. The reaction mixture was incubated at 90˚C for 30 min, with continuous mixing. An aliquot of 10 mM diethylenetriamine tetra-acetic acid (DTPA), pH 6.0, was then added to a final concentration of 1 mM, and the reaction mixture was mixed and incubated at room temperature for 5 min. The radiolabeled conjugate was purified by RP-HPLC, using a gradient of 0-50% solvent B (solvent A: 0.1% TFA/H_2O; solvent B: 0.1% TFA/CH_3CN) in 30 min. Typical radiolabeling and radiochemical purity yields were 92% or better (Figure 2). Purified fractions were concentrated *in vacuo* to remove organic solvents. Then the pH was adjusted to approximately 5.0 with 0.1 M NaOH, and the solution was diluted with normal saline for addition to the cellular suspension. DOTA-Tyr^3-octreotate was labeled with ^{177}Lu as follows: 37 MBq of $^{177}LuCl_3$ in 150 μl of 30 mM sodium acetate/25 mM sodium ascorbate, pH 5.0, was incubated with 1 μg of DOTA-Tyr^3-octreotate at 99˚C for 30 min. Radiometal incorporation and radiochemical purity (typically 98%) were determined by radio-thin-layer chromatography (radio-TLC) (22). The radiolabeled peptide was diluted with normal saline for addition to the cellular suspension without further purification.

Cell culture. The human non-Hodgkin's lymphoma cell line Mec-1 (DSMZ Cell Lines Bank, Braunschweig, Germany) was cultured in a modified RPMI-1640 medium containing 1 mM sodium pyruvate, 10 mM HEPES, 2 mM L-glutamine, 0.4 mg/ml gentamicin and supplemented with 10% heat inactivated fetal bovine serum at 37˚C in a 5% CO_2 atmosphere. Cell passage numbers were deliberately kept below 10, as unpredictable growth and large-scale colony death were observed in flasks containing cells with passage numbers greater than 15. Passage numbers fewer than 10 were consistently monitored to maintain cellular viability and predictable confluent growth periods. All cell culture methodologies were based on the work of Jia *et al*. (19).

Cell uptake and efflux assays. The methods for cell uptake and efflux were based on the previously published techniques of Jia *et al*. and Lewis *et al*. (19, 21). For uptake studies, aliquots of 37 kBq of the ^{177}Lu-labeled conjugates were added to 1×10^7 Mec-1 cells in 5 ml of serum-free media. During incubation at 37˚C and 5% CO_2, triplicate aliquots were removed at various time points from 1 min to 4 h. Cells were isolated by centrifugation, washed, and counted separately from the liquid phase to determine the percentage uptake of the radiopharmaceutical. The cell-associated radioactivity in each sample phase (pellet, supernatant, and wash) was counted on a gamma counter. Uptake was then calculated by dividing the pellet-associated radioactivity by the total radioactivity. Cell viability after

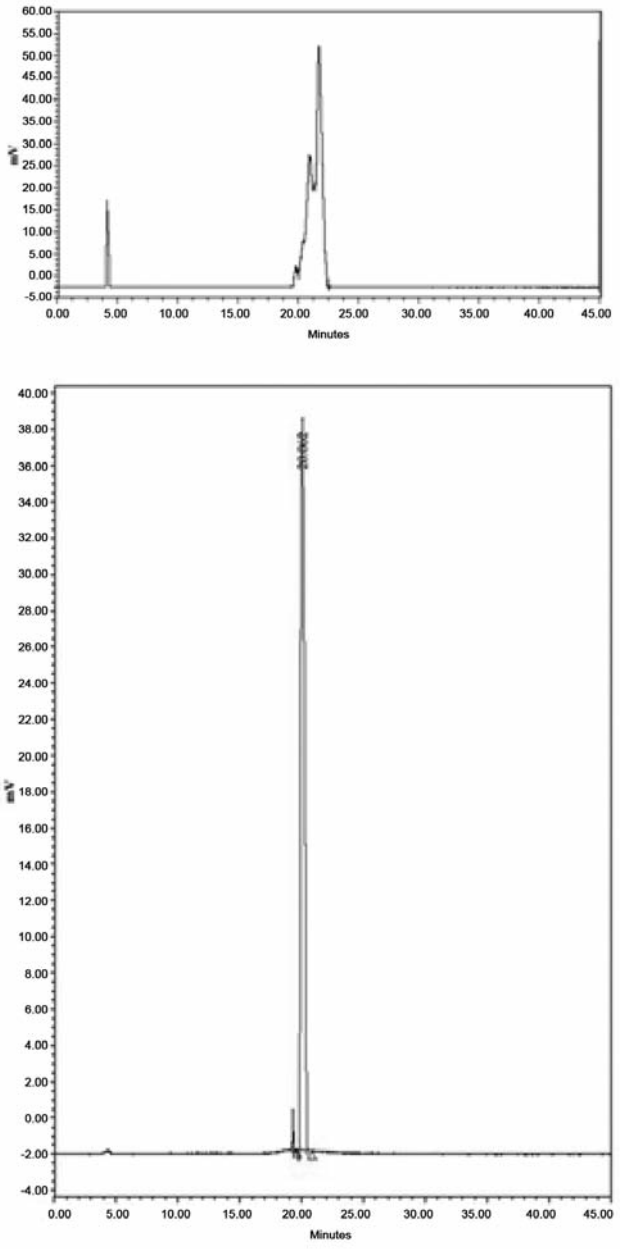

Figure 2. *RP-HPLC chromatograms of the crude (top) and peak purified (bottom) ^{177}Lu-labeled PNA compound.*

4 h of incubation was determined to be ~98% by Trypan Blue (Thermo Fisher Scientific, Waltham, MA, USA) exclusion and hemacytometry.

For efflux studies, radiopharmaceutical uptake was performed as described above for 2 h at 37˚C and 5% CO_2. Cells were then isolated by centrifugation, washed with fresh medium to remove residual radioactivity, and re-suspended in fresh medium. Efflux of ^{177}Lu from the cells was measured at subsequent time points from 1 min to 4 h, after isolating aliquots of the cell pellets and counting the radiation in them separately from the supernatants and washes, as described above.

Radiation dosimetry. The radiation-absorbed dose delivered by the ^{177}Lu-labeled PNA–peptide conjugate was calculated using a Monte Carlo 'n' particle model (MCNP5) (23). This Fortran-based Monte Carlo projection uses multiple iterations of random modeled decay events to estimate the dose to the nucleus or cytoplasm based on the location of the decay and the geometry of the total closed system. The assumptions for this model were that the Mec-1 cells have an average radius of 4 μm, a nuclear volume of approximately 40%, and a cell volume fraction of 0.15% of the total water matrix. An MCNP model was calculated using the β^- emission of ^{177}Lu. Due to the planar arrangement of the cells in the wells of plates, the γ emission of ^{177}Lu was not taken into account. The dosimetry calculations were used to normalize the absorbed doses of the radiopharmaceuticals (24, 25).

Proliferation and viability assays. Cells were counted daily using the Alamar Blue (Invitrogen, Carlsbad, CA, USA) assay on a fluorescence spectrometer operated at 530 nm/590 nm. Cells were also counted daily on a hemacytometer. The non-viable fraction was recorded after the cells were diluted with 0.4% Trypan Blue vital stain as per the manufacturer's instructions. Dilutions were appropriate to yield a minimum 100 cell count. Three replicates allowed detection of a 25% difference with a statistical power of 0.80 and an α value of 0.05. Results from the Alamar Blue assay were validated to within 2% of those of conventional hemacytometry.

During the viability and proliferation assays, the amount of each compound added was varied as a function of mass and the radiation dose administered. Doses of 0, 0.25, 0.50, 1.0, and 2.0 Gy and from 0, 2, 5, and 10 μg of compound, respectively, were evaluated. Comprehensive studies (n=3) were conducted for each experimental category. Each well in a sterile 24-well plate was inoculated with 4×10^5 Mec-1 cells, incubated in 1 ml of medium, and treated according to its respective experimental category. Cellular response was monitored using Alamar Blue, as well as Trypan Blue exclusion and hemacytometry.

Statistical analysis. Utilizing a two-way analysis of variance (ANOVA) without replication in conjunction with a randomized complete block design for the study allowed for the collection and analysis of sufficient data by treating each well as a distinct block in the treatment instead of as a block replicate. Furthermore, since the samples were seeded from the same original source, it was possible to pool the data and manipulate by treatment type and day.

The use of a *post-hoc* comparison of effects by a Bonferroni test of significant difference allowed for the resolution of effects both within and between treatments or study conditions.

Results

Cellular uptake and efflux assays were used to determine the amount of the PNA compound that was residualized intracellularly. The results of these assays are shown in Figure 3 A and B. The uptake results displayed the anticipated second-order shaped curve indicative of a cellular saturation process, with maximal uptake values reaching 2.5% of the total radioactivity at 4 h. The efflux data showed that by the 4-h time point, 30% of the compound was still retained in the tumor cells. An integration of uptake and

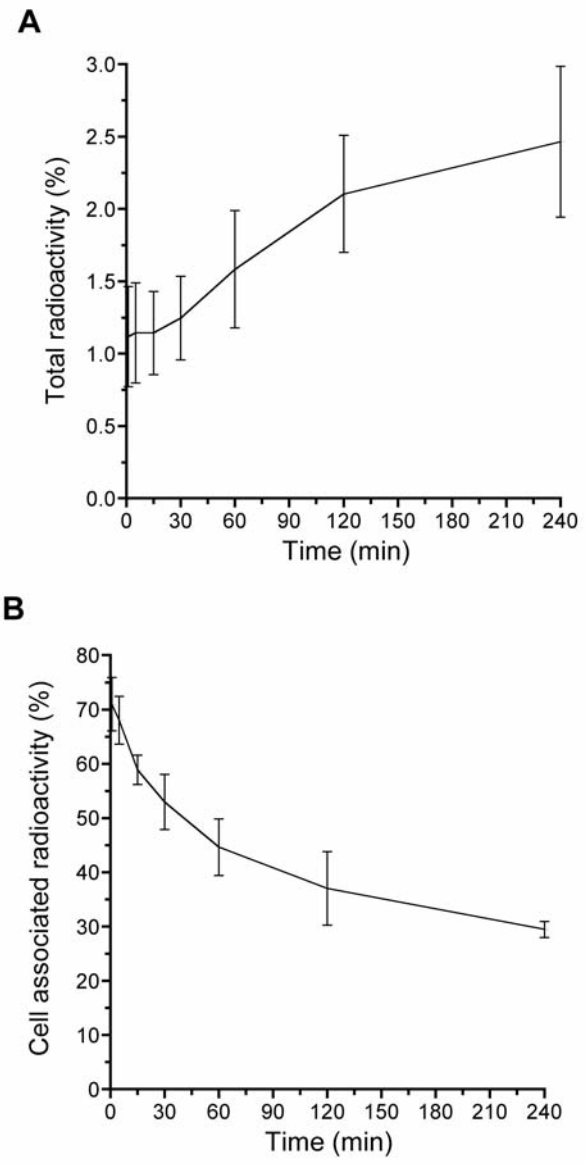

Figure 3. *Mec-1 cell uptake (A) and efflux (B) of PNA compound over a 4 h period.*

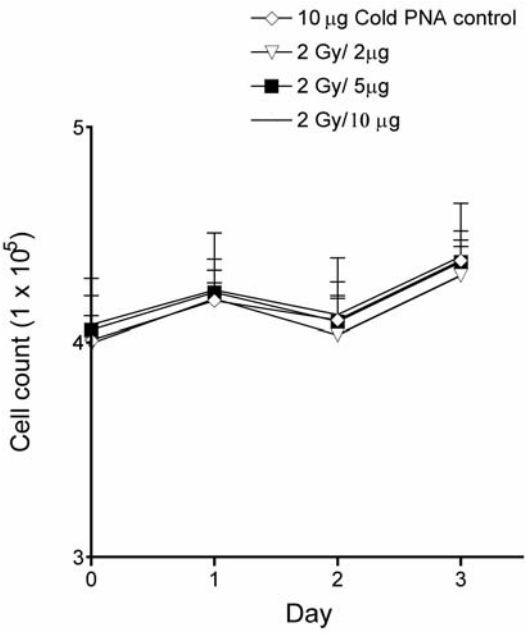

Figure 4. *Mec-1 cell proliferation after treatment with 10 μg of ^{177}Lu-labeled BCL2 antisense PNA–Tyr^3-octreotate at 0.25, 0.50, 1.0, and 2.0 Gy absorbed doses, compared to 10 μg of unlabeled PNA–peptide.*

efflux data over time allowed dosimetry calculations to be made utilizing an MCNP5 projection. Incorporating the values calculated by the program into the following equation:

$$Decay = \frac{Act_o(Bq)}{\lambda(s^{-1})}(1 - e^{-\lambda t}),$$

where Act_0 (in Bq) is the initial radioactivity in the volume of interest, λ (in s^{-1}) is the decay constant (=ln2/$T_{1/2}$), and t is the elapsed time from initiation of the study. This equation allowed for calculation of self and cross doses from nucleus, cytoplasm, and water matrix, respectively (Table I). This analysis also allowed for the calculation of the number of kBq of ^{177}Lu required to prepare test conditions of 0.25, 0.5, 1.0, and 2.0 Gy (Table I). Additionally, masses of 2, 5, and 10 μg of the PNA–peptide were tested for optimal effect. Choice of PNA masses was made based on internalization potential relative to the number of somatostatin cell surface receptors. Mass- and dose-variable proliferation and viability studies were designed to evaluate the optimal combination of radiation-absorbed dose and mass of antisense PNA compound, in an effort to affect tumor cell death *via* radiologic and biologic means, respectively.

Assays designed to assess the proliferative effects of varied PNA mass and ^{177}Lu absorbed dose of the labeled PNA–peptide conjugate yielded no statistically significant results (Figure 4). However, viability assays of varied PNA mass and ^{177}Lu absorbed dose of the labeled PNA–peptide conjugate indicated mass- and dose-dependent responses. The effects on cellular viability were graphically observed in the stratification of the data, with the mass of PNA administered correlating to the observed level of decrease. This effect was most noticeable in the 2 Gy/10 μg test condition, although also observed to a lesser extent at the lower absorbed dose/PNA mass test conditions of ^{177}Lu-PNA-peptide (Figure 5). At 2 Gy/10 μg, the percentage of viable cells decreased by 35.8% from day 0 to day 3, as compared to the 24.2% reduction in viability over the same time period with 10 μg of unlabeled PNA–peptide. Statistical

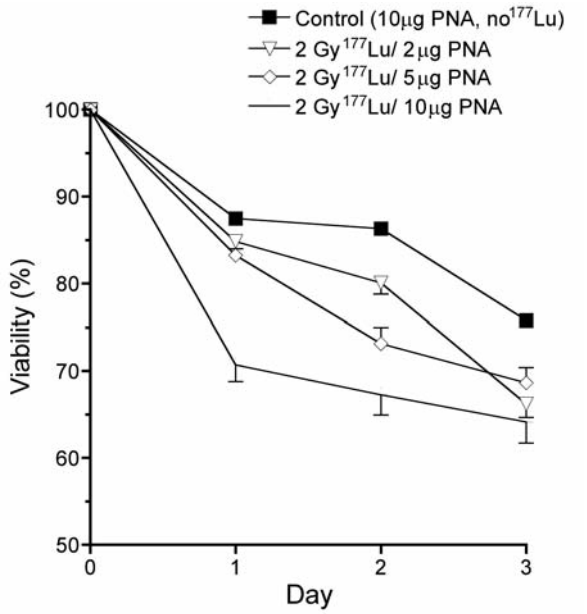

Figure 5. *Mec-1 cell viability after treatment with 2, 5, and 10 μg of ^{177}Lu-labeled BCL2 antisense PNA–Tyr^3-octreotate at an absorbed dose of 2.0 Gy, compared to 10 μg of unlabeled PNA–peptide.*

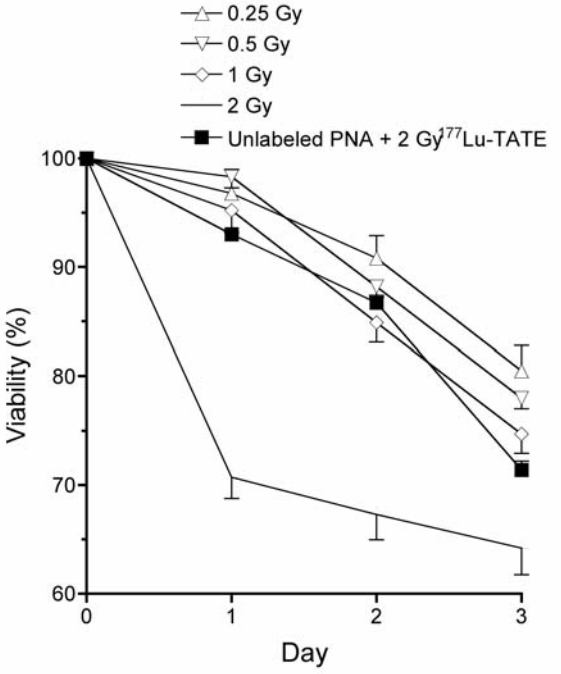

Figure 6. *Mec-1 cell viability after treatment with 10 μg of ^{177}Lu-labeled BCL2 antisense PNA–Tyr^3-octreotate at absorbed doses of 0.25, 0.5, 1.0, and 2.0 Gy, compared to the combination of 10 μg of unlabeled PNA–peptide and ^{177}Lu-Tyr^3-octreotate (TATE), administered at an absorbed dose of 2.0 Gy.*

analysis of the observed reduction in viability, relative to mass and dose administered, indicated a significant difference when compared to the unlabeled PNA–peptide control (day 1 $p<0.003$, day 2 $p<0.005$, day 3 $p<0.002$).

Additional results also suggest an additive relationship between the observed efficacy of the mass of PNA and the dose of radiological insult administered, as compared to the combination of unlabeled PNA–peptide and ^{177}Lu-Tyr^3-octreotate (Figure 6). When treated with 2 Gy/10 μg of the ^{177}Lu-labeled PNA–peptide, the percentage of viable cells decreased by 35.8% from day 0 to day 3, as compared to the 28.6% reduction in viability appreciated in the combination of unlabeled PNA–peptide and ^{177}Lu-DOTA-Tyr^3-octreotate. Statistical analysis indicated a significant difference (day 1 $p<0.005$, day 2 $p<0.004$, day 3 $p<0.002$) for the 2 Gy/10 μg test condition, providing confirmation of the additive relationship.

Discussion

Leveraging the anti-apoptotic functionality of the *BCL2* proto-oncogene along with the overexpression of SSTR2 in human NHL allowed us to demonstrate the feasibility of a novel site-directed therapeutic agent. A biological effect was achieved using somatostatin peptide analogues as delivery vehicles for an anti-*BCL2* agent in receptor mediated uptake, resulting in a specific antisense effect inhibiting the translation of *BCL2* mRNA and thus down-regulating the anti-apoptotic function of the protein (19). In the present work, a radiological insult was achieved by labeling the PNA–peptide conjugate with cytotoxic lutetium-177, with a primary β^- emission of 497 keV.

Uptake and efflux assays demonstrated that the compound was retained in Mec-1 cells after 4 h of elapsed time. Administration of the PNA–peptide conjugate at 2 Gy and in varying masses showed a direct correlation between cell death and the mass of PNA–peptide administered, with the most significant effect being observed with the largest tested mass, 10 μg. Likewise, a direct correlation was observed between cell death and the radiation-absorbed dose of ^{177}Lu administered.

Proliferation assays did not show a generalized decrease in cellular proliferation over the course of the study in each test condition. Thus, the statistically insignificant results generated may have been the result of a different causal relationship. We believe that the different effects on proliferation and vialbility were actually due to different cellular responses. Cell death following internal emitter therapy often results primarily in apoptotic, as opposed to necrotic, cell death. It is expected that apoptosis would occur over the course of several days under the conditions employed, affecting the observed viability of the colony as opposed to its proliferation over a three-day

Table I. *Table of dosimetric calculations based on values obtained from the MCNP5 projection analysis of uptake and efflux data.*

Location of Lu-177	Dose to nucleus (Gy/decay)	Dose to cytoplasm (Gy/decay)	Relative dose (Gy)	Activity (kBq)	Activity (μCi)
Nucleus	0.0021	0.0008	0.25	1.57×10^{-2}	4.25×10^{-4}
Cytoplasm	0.0008	0.0012	0.5	3.14×10^{-2}	8.50×10^{-4}
Water matrix outside of the cell	0.0011	0.0013	1	6.29×10^{-2}	1.70×10^{-3}
Total dose per decay (Gy)	0.004	0.0033	2	1.26×10^{-1}	3.40×10^{-3}

period. The observation of any proliferative effects resulting from necrotic cell death would have necessitated a dramatic increase in the length of time that the study was allowed to run. This was counterproductive to the determination of reproducible results, as the physical dimensions or maximum volume of each well dictates the length of time that the seeded cells remain viable.

The observation that an increase in the mass of PNA administered translated to an increased antisense effect or mass/dose response was most readily associated with the 10 μg/2 Gy test condition. These results led to the observation that combined effects of ^{177}Lu radiotherapy with *BCL2* antisense therapy acted with strongly additive characteristics in reducing viability, as compared to the combination of unlabeled PNA–peptide and ^{177}Lu-Tyr^3-octreotate.

One plausible hypothesis as to the source of the observed additive effect can be derived from the characteristics of the ^{177}Lu radionuclide as it decays to ^{177}Hf. As previously discussed, the predominent decay emmision of ^{177}Lu is a β^- particle; however, there are two associated γ emissions with respective energies of 113 keV and 208 keV. Newly reported data suggest the emission of an average of two internal conversion (IC) electrons as a commonly utilized alternative branch to the 113 keV γ emission (26). We have demonstrated a measurable reduction in cellular viability with the administration of the ^{177}Lu-Tyr^3-octreotate, which is only capable of targeting SSTR2 surface receptors. We attribute the reduction in cellular viability caused by this agent to DNA damage caused by the β^- particle emission. Furthermore, our data have demonstrated that the unlabeled PNA–peptide has some effect on viability reduction which is due to the direct biological action of the compound. One hypothesis generated to explain the observed additive effect of the radiolabeled PNA–peptide conjugate relative to its constituent parts is that the observed additive effect is due to the intracellular emission of IC electrons.

We have considered that the *BCL2* mRNA is found in the cytosol and translation occurs *via* action of the ribosome. Furthermore, much of the ^{177}Lu-labeled PNA–peptide conjugate would be selectively bound to BCL2 mRNA. Given the characteristicly short path length and high linear energy transfer of an IC electron, if one were released while the mRNA was undergoing translation, three plausible scenarios present themselves. Firstly, the IC electron could strike the mRNA, rendering the strand irreparably damaged. Translation of the strand would then stop and the overall concentration of *BCL2* protein would be reduced. Secondly, the emitted IC electron might damage a developing amino acid sequence in active translation, rendering it non-functional. If the protein is non-functional then its overall anti-apoptotic effect would be diminished. Thirdly, the emitted IC electron may damage the ribosome itself while engaged in active translation of the *BCL2* mRNA. This effect could have several possible outcomes, including an inability to produce not only the *BCL2* protein, but also many other proteins necessary for cell survival. Regardless, the result is an observed decrease in cellular viability.

The current series of experiments has displayed definite promise for the development of a corresponding multimodality agent for targeted radiotherapy with a combined antisense effect. Work is currently underway to evaluate the ^{177}Lu-labeled anti-*BCL2*–PNA–peptide agent in a tumor-bearing mouse model.

Acknowledgements

Authors acknowledge the Department of Veterans Affairs, for providing facilities and resources at the Harry S. Truman Memorial Veterans' Hospital in Columbia, MO, USA. This work was funded by grants from the Department of Veterans Affairs (Grant Number BX000575, M.R.L.) and from the Society of Nuclear Medicine (SNM Student Fellowship Award, E.R.B.). The Authors would also like to acknowledge Dr. Fabio Gallazzi for the synthesis of the PNA–peptide conjugate and thank Dr. Charles W. Caldwell for providing the Mec-1 cell line.

References

1 Hermine O, Haioun C, Lepage E, d'Agay MF, Briere J, Lavignac C, Fillet G, Salles G, Marolleau JP, Diebold J, Reyas F and Gaulard P: Prognostic significance of *BCL2* protein expression in aggressive non-Hodgkin's lymphoma. Blood *87*: 265-272, 1996.

2 Hill M, MacLennan K and Cunningham D: Prognostic significance of *BCL2* expression and *BCL2* major breakpoint rearrangement in diffuse large cell non-Hodgkin's lymphoma: a British National Lymphoma Investigation study Blood *88*: 1046-1051, 1996.

3 Milner A, Grand R, Vaughn A, Armitage R and Gregory C: Differential effects of *BCL2* on survival and proliferation of human B-lymphoma cells following gamma-irradiation. Oncogene *15*: 1815-1823, 1997.
4 Mirkovic N, Voehringer D, Story M, McConkey D, McDonnell T and Meyn R: Resistance to radiation-induced apoptosis in *BCL2*-expressing cells is reversed by depleting cellular thiols. Oncogene *15*: 1461-1471, 1997.
5 Tsujimoto Y, Cossman J, Jaffe E and Croce C: Involvement of the *BCL2* gene in human follicular lymphoma. Science *228*: 1440-1444, 1985.
6 Reubi JC, Waser B, Van Hagen M, Lamberts SWJ, Krenning EP, Gebbers JO and Laissue AJ: *In vitro* and *in vivo* detection of somatostatin receptors in human malignant lymphomas. Int J Cancer *50*: 895-900, 1992.
7 Bernhardt P, Oddstig J, Kolby L, Nilsson O, Ahlman H and Forssell-Aronsson E: Effects of treatment with ^{177}Lu-DOTA-Tyr^3-octreotate on uptake of subsequent injection in carcinoid-bearing nude mice. Cancer Biother Radiopharm *22*: 644-653, 2007.
8 Bugaj JE, Erion JL, Johnson MA, Schmidt MA and Srinivasan A: Radiotherapeutic efficacy of ^{153}Sm-CMDTPA-Tyr^3-octreotate in tumor-bearing rats. Nucl Med Biol *28*: 327-334, 2001.
9 Kwekkeboom DJ, Bakker WH and Kam BL: Treatment of patients with gastro-entero-pancreatic (GEP) tumors with the novel radiolabeled somatostatin analogue [^{177}Lu-DOTA-Tyr^3]octreotate. Eur J Nucl Med Mol Imaging *30*: 417-422, 2003.
10 Kwekkeboom DJ, Teunissen JJ, Bakker WH, Kooij PP, de Herder WW, Feelders RA, van Eijck CH, Esser JP, Kam BL and Krenning EP: Radiolabeled somatostatin analogue [^{177}Lu-DOTA-Tyr^3]octreotate in patients with endocrine gastroenteropancreatic tumors. J Clin Oncol *23*: 2754-2762, 2005.
11 Lewis JS, Srinivasan A, Schmidt MA and Anderson CJ: *In vitro* and *in vivo* evaluation of ^{64}Cu-TETA-Tyr3-octreotate. a new somatostatin analog with improved target tissue uptake. Nucl Med Biol *26*: 267-273, 1999.
12 Lewis JS, Wang M, Laforest R, Wang F, Erion JL, Bugaj JE, Srinivasan A and Anderson CJ: Toxicity and dosimetry of ^{177}Lu-DOTA-Y3-octreotate in a rat model. Int J Cancer *94*: 873-877, 2001.
13 Kirin SI, Ott I, Gust R, Mier W, Weyhermuller T and Metzler-Nolte N: Cellular uptake quantification of metalated peptide and peptide nucleic acid bioconjugates by atomic absorption spectroscopy. Angew Chem Int Ed Engl *47*: 955-959, 2008.
14 Mier W, Eritja R, Mohammed A, Haberkorn U and Eisenhut M: Peptide PNA conjugates: targeted transport of antisense therapeutics into tumors. Angew Chem Int Ed Engl *42*: 1968-1971, 2003.
15 Teunissen JJ, Kwekkeboom DJ, Kooij PPM, Bakker WH and Krenning EP: Peptide receptor radionuclide therapy for non-radioiodine-avid differentiated thyroid carcinoma. J Nucl Med *46*: 107S-114S, 2005.
16 Valkema R, Pauwels SA, Kvols LK, Kwekkeboom DJ, Jamar F, de Jong M, Barone R, Walrand S, Kooij PPM, Bakker WH, Lasher J and Krenning EP: Long-term follow-up of renal function after peptide receptor radiation therapy with ^{90}Y-DOTA^0,Tyr^3-octreotide and ^{177}Lu-DOTA^0, Tyr^3-octreotate. J Nucl Med *46*: 83S-91S, 2005.
17 de Jong M, Breeman WAP, Valkema R, Bernard BF and Krenning EP: Combination radionuclide therapy using ^{177}Lu- and ^{90}Y-labeled somatostatin analogs. J Nucl Med *46*: 13S-17S, 2005.
18 Muller C, Forrer F, Bernard BF, Melis M, Konijnenberg M, Krenning EP and de Jong M: Diagnostic *versus* therapeutic doses of [^{177}Lu-DOTA-Tyr^3]-octreotate: uptake and dosimetry in somatostatin receptor-positive tumors and normal organs. Cancer Biother Radiopharm *22*: 151-159, 2007.
19 Jia F, Daibes-Figueroa S, Gallazzi F, Balaji BS, Hannink M, Lever SZ, Hoffman TJ and Lewis MR: Molecular imaging of *BCL2* expression in small lymphocytic lymphoma using ^{111}In-labeled PNA-peptide conjugates. J Nucl Med *49*: 430-438, 2008.
20 Gallazzi F, Wang Y, Jia F, Shenoy N, Landon LA, Hannink M, Lever SZ and Lewis MR: Synthesis of radiometal-labeled and fluorescent cell-permeating peptide-PNA conjugates for targeting the *BCL2* proto-oncogene. Bioconjug Chem *14*: 1083-1095, 2003.
21 Lewis MR, Jia F, Gallazzi F, Wang Y, Zhang J, Shenoy N, Lever SZ and Hannink M: Radiometal-labeled peptide PNA conjugates for targeting *BCL2* expression: preparation, characterization, and *in vitro* mRNA binding. Bioconjug Chem *13*: 1176-1180, 2002.
22 Lewis JS, Lewis MR, Cutler PD, Srinivasan A, Schmidt MA, Schwarz SW, Morris MM, Miller JP and Anderson CJ: Radiotherapy and dosimetry of ^{64}Cu-TETA-Tyr^3-octreotate in a somatostatin receptor-positive, tumor-bearing rat model. Clin Cancer Res *5*: 3608-3616, 1999.
23 Miller WH, Hartmann-Siantar C, Fisher D, Descalle M-A, Daly T, Lehmann J, Lewis MR, Hoffman T, Smith J, Situ PD and Volkert WA: Evaluation of beta-absorbed fractions in a mouse model for ^{90}Y, ^{188}Re, ^{166}Ho, ^{149}Pm, ^{64}Cu, and ^{177}Lu radionuclides. Cancer Biother Radiopharm *20*: 436-449, 2005.
24 Balkin ER, Jia F, Miller WH and Lewis MR: *In vitro* evaluation of ^{177}Lu antisense radiotherapy. Trans Am Nucl Soc *98*: 850-851, 2008.
25 Bryan JN, Jia F, Mohsin H, Sivaguru G, Miller WH, Anderson CJ, Henry CJ and Lewis MR: Comparative uptakes and biodistributions of internalizing *vs*. noninternalizing copper-64 radioimmunoconjugates in cell and animal models of colon cancer. Nucl Med Biol *32*: 851-858, 2005.
26 Deepa S, Vijay Sai K, Gowrishankar R, Dwarakarani R and Venkataramaniah K: Precision electron-gamma spectroscopic measurements in the decay of ^{177}Lu. Appl Radiat Isotopes *69*: 869-874, 2011.

Received July 8, 2011
Revised August 22, 2011
Accepted August 23, 2011