DOE/ER/61459-4

Oliver W. Press M.D. Ph.D. August 15, 1996

OHER Nuclear Medicine Research Final Technical Report

A. PROJECT TITLE:

Improved Radioimmunotherapy of Hematologic Malignancies

DOE Grant #:	DE-FG06-92ER61459
Principal Investigator:	Oliver W. Press MD PhD
Organization:	University of Washington
Address:	Mailstop RC-08 Pacific Ave. Seattle, WA 98195
Telephone:	206-548-4938 (FAX = 206-548-4509)

B. PATENTABLE INVENTIONS: Not Applicable

C. PROGRESS REPORT

DOE grant DE-FG06-92ER61459 entitled "Improved Radioimmunotherapy of Hematologic Malignancies" has generated 17 publications, 11 abstracts, and 37 presentations at national and international meetings as listed below.

Editorials Featuring Our Research

1. Hall SS. Monclonal Antibodies at age 20: Promise at last? *Science* 270: 915, 1995.
2. McNeil C. A new generation of monoclonal antibodies arrives at the clinic. *J. National Cancer Inst.* 87: 1658-1659, 1995.
3. Thielman J. Antibodies pack punch to destroy cancer. *Science in the National Interest*, Office of Energy Research, Department of Energy, Volume 1 (Issue 3), May, 1995.

Publications Supported by DOE grant DE- FG06-92ER61459 between 1992-95:

1. Press OW, Eary J, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, Glenn S, Butchko G, Fisher D, Porter B, Matthews D, Fisher L, Bernstein ID. Radiolabeled antibody therapy of B cell lymphomas with autologous bone marrow support. *New Engl J Med* 324: 1219-1224, 1993.
2. Press OW, Eary JF, Appelbaum FR, Martin PJ, Nelp WB, Glenn S, Fisher DR, Porter B, Matthews DC, Gooley T, Bernstein ID. A Phase II trial of ^{131}I-B1 (anti-CD20) antibody therapy with autologous stem cell transplantation for relapsed B cell lymphomas. *Lancet* 346: 336-340, 1995.
3. DeSantes K, Slamon D, Anderson SK, Shepard M, Fendly B, Maneval D, Press OW. Radiolabeled antibody targeting of the HER-2/*neu* oncoprotein. *Cancer Res* 52: 1916-1923, 1992.
4. Shan D and Press OW. Constitutive endocytosis and degradation of CD22 by human B-cells. *J. Immunol.* 154: 1-10, 1995.
5. Press OW, Howell-Clark J, Anderson S, Appelbaum FR, Bernstein ID. Retention of B-cell-specific monoclonal antibodies by human lymphoma cells. *Blood* 83: 1390-1397, 1994.
6. Geissler F, Anderson SK, Venkatesan P, Press OW. Intracellular catabolism of radiolabeled anti-μ antibodies by malignant B-cells. *Cancer Res* 52: 2907-2915, 1992.
7. Grossbard M, Press OW, Appelbaum F, Bernstein I, Nadler L. Monoclonal antibody-based therapies of leukemia and lymphoma. *Blood* 80: 863-878, 1992.
8. Press OW, Eary J, Appelbaum F, Badger C, Bernstein I. Radioimmunoconjugate therapy of malignant lymphomas. In: Dana B, ed. Malignant Lymphomas. Boston: Kluwer Academic Publishers, 1993, 127-145.

9. Press OW, Eary J, Appelbaum F, Bernstein I. High-dose radioimmunotherapy of lymphomas. In: Rosen S, ed. Immunoconjugate Therapy of Hematologic Malignancies. Norwell, MA: Kluwer Academic Publishers, 1993, 13-22.
10. Press OW, Eary JF, Appelbaum FR, Bernstein ID. Radiolabeled antibody therapy of lymphomas. In: DeVita VT, S Hellman S, Rosenberg SA, eds. *Biologic Therapy of Cancer Updates V.* Philadelphia: JB Lippincott Co., 1994, 1-13.
11. Press OW, Eary JF, Appelbaum, FR, Bernstein ID. Treatment of relapsed B-cell lymphomas with high-dose radioimmunotherapy and bone marrow transplantation. In: Goldenberg DM, ed. Cancer Therapy with Radiolabeled Antibodies. Boca Raton, FL: CRC Press, Inc., 1994, 229-237.
12. Press OW, Appelbaum FR, Eary JF, Bernstein ID. Radiolabeled antibody therapy of lymphomas. In: DeVita VT, Hellman S, Rosenberg SA, eds. Important Advances in Oncology. Philadelphia: JB Lippincott Company, 1995, 157-171.
13. Press OW, Eary JF, Appelbaum FR, Bernstein ID. Monoclonal antibody targeting of radioactive isotopes for relapsed lymphomas. In: Buckner CD, ed. *Technical and Biological Components of Marrow Transplantation.* Norwell, MA: Kluwer Academic Publishers, 1995, 281-297.
14. Press OW. Treatment of recurrent lymphomas with unmodified antibodies and radioimmunoconjugates. *Tumor Targeting* 1: 31-35, 1995.
15. Press OW, Wijdenes J, Glennie MJ, Bagshawe KD. Targeted therapy of cancer and autoimmune diseases. *Pharmacology and Therapeutics,* (in press) 1995.
16. Press OW and Bilge A. Immune conjugates in immunotherapy. In: Kipps TJ, ed. Handbook of Experimental Immunology. Blackwell Scientific Publications, (in press), 1995.
17. Press OW, Shan D, Howell-Clark J, Eary J, Appelbaum F, Matthews D, Hinman L, Shochat D, and Bernstein ID. Comparative metabolism and retention of Iodine-131, Yttrium-90, and Indium-111 radioimmunoconjugates by cancer Cells. (submitted).

Abstracts Supported by DOE grant DE- FG06-92ER61459 between 1992-95

1. Press OW, Eary JF, Badger CC, Martin PJ, Appelbaum FR, Levy R, Miller R, Nelp WB, Fisher DR, Wiseman G, Matthews D, Bernstein ID. Radiolabeled antibody therapy followed by autologous marrow transplantation for relapsed B cell lymphomas. *J Cellular Biochem* (Suppl 16A): 204, 1992.
2. Press O, Eary J, Badger C, Appelbaum F, Martin P, Wiseman G, Nelp W, Fisher DR, Miller R, Porter B, Matthews D, Bernstein I. Radiolabeled antibody (RAb) therapy of relapsed B cell lymphomas. *Proc Am Soc Clin Onc* 11: 318, 1992.
3. Press OW, Eary JF, Badger CC, Martin PJ, Appelbaum FR, Nelp WB, Fisher DR, Matthews D, Bernstein ID. Radiolabeled antibody therapy of B cell lymphomas. *J Cellular Biochem Suppl* 17E: 271, 1993.
4. Press OW, Eary JF, Martin PJ, Appelbaum FR, Glenn SD, Butchko GM, Matthews DC, Bernstein ID. Phase I and II trials of Iodine-131-labeled anti-CD20 (B1) antibody therapy for relapsed B cell lymphomas. *Blood* 82: 333a, Suppl 1, 1993.
5. Press OW, Eary JF, Martin PJ, Appelbaum FR, Glenn SD, Butchko GM, Matthews DC, Bernstein ID. Radioimmunotherapy of B cell lymphomas with Iodine-131-labeled anti-CD20 (B1) monoclonal antibodies. In: Proceedings of the 8th NCI-EORTC Symposium on New Drugs in Cancer Therapy. *Annals Oncol* 5: 70, Suppl 5, 1994.
6. Bilge A, Press OW. Translocation of ricin A-chain (RTA) into vesicles reconstituted from purified golgi and endoplasmic reticulum (ER). *FASEB J* 8: A504, 1994.
7. Press O, Eary J, Martin P, Appelbaum FR, Glenn S, Butchko G, Matthews D, Bernstein ID. Radiolabeled antibody therapy of lymphomas. *Can J Physiology and Pharm* 72: 49, Suppl. 1, 1994.
8. Press OW, Eary JF, Martin PJ, Appelbaum FR, Nelp WB, Matthews DC, Bernstein ID. Preliminary results of a Phase II trial of Iodine-131-labeled anti-CD20 (B1) antibody therapy

with bone marrow rescue for patients with relapsed B-Cell lymphomas. *J Immunotherapy* 16: 160, 1994.

9. Zhan JB, Stayton P, and Press OW. Manipulating the intracellular trafficking of ricin a chain by addition of organelle-specific retention signals to its carboxyl terminus. *Proc Fourth Int Symposium on Immunotoxins*, Myrtle Beach, 1995.
10. Press O, Eary J, Martin P, Appelbaum F, Nelp W, Matthews D, Fisher D, Glenn S and Bernstein I. A phase II trial of Iodine-131-labeled anti-CD20 (B1) antibody therapy with bone marrow transplantation for relapsed non-Hodgkin's lymphomas. *Proc. Am. Soc. Clin. Oncol.* 14: 388, 1995.
11. Press OW. High dose radioimmunotherapy of relapsed B cell lymphomas. *Bioconjugate Chemistry* (in press), 1995.

Invited Presentations at National and International Meetings Concerning Research from this Grant:

1. Invited presentation to the NCI National Cancer Advisory Board, September 18-21, 1993, Washington DC.
2. Plenary speaker at the 8th NCI-EORTC Symposium on New Drugs in Cancer Therapy, Amsterdam, The Netherlands, March 12-19, 1994.
3. Featured speaker at the Tufton Charitable Trust Conference, The Royal Society of Medicine, London, England, October 10-11, 1994 .
4. Plenary speaker at the 11th International Hammersmith Conference on the Advances of Applications of Monoclonal Antibodies in Clinical Oncology, Lesvos, Greece, May 23-26, 1994.
5. Plenary speaker for the 9th International Conference of Monoclonal Antibody Immunoconjugates, San Diego, CA, March 3-5, 1994.
6. Invited speaker at the 12th International Congress of Pharmacology, Montreal, Canada, July 26-28, 1994.
7. Presentations to legislators from the states of Washington, Alaska, Montana, and Idaho (WAMI Legislative Conference), November 29-30, 1993, and March 28, 1994.
8. Fifth Conference on Radioimmunodetection and Radioimmunotherapy of Cancer, Princeton, NJ, October 6-8, 1994.
9. Federation of Associated Societies for Experimental Biology and Medicine (FASEB), Anaheim, CA, April 24-28, 1994.
10. Presentation at the 35th Annual Meeting of the American Society of Hematology, St. Louis, MO, December 3-7, 1993.
11. Presentation at the 1995 37th Annual Meeting of the American Society of Hematology, Seattle, December 2-6, 1995.
12. American Society of Clinical Oncology 29th Annual Meeting, (ASCO), Orlando, FL, May 16-18, 1993.
13. American Society of Clinical Oncology (ASCO), 30th Annual Meeting, Dallas, TX, May 14-17, 1994.
14. American Society of Clinical Oncology, 5th Annual Fall Educational Conference, Atlanta, GA, November 11-13, 1994.
15. American Society of Clinical Oncology, 31st Annual Meeting, Los Angeles, CA, May 20-23, 1995.
16. Society for Biologic Therapy 10th Annual Scientific Meeting, Williamsburg, VA, November 1-4, 1995.
17. Cancer Research Institute 1995 International Symposium on Monoclonal Antibodies & Cancer Therapy, New York, NY, October 16-18, 1995.
18. Central Oregon Oncology Conference on New Approaches to B Lymphocyte Malignancies, Bend, OR, January 24-26, 1992.
19. Denali Oncology Group Conference, Circle Hot Springs, AK, June 17-18, 1993.
20. Plenary speaker for the 9th Annual Cancer Symposium on Advances In The Biological Therapy of Cancer, Providence Medical Center, Portland, OR, April 29, 1994.

21. Plenary speaker at Puget Sound Oncology Consortium Symposium on Marrow Transplantation, Seattle, WA, April 30, 1994.
22. Keystone Symposium on B and T Cell Lymphomas, Copper Mountain, CO, April 17-23, 1993.
23. Grand Rounds lectures at Harvard Collaborative Oncology Group Symposium on Non-Hodgkin's Lymphoma, Brigham and Women's Hospital, and Dana Farber Cancer Institute, Boston, MA, April 27, 1995.
24. Grand Rounds lectures at UCLA Medical Center and Olive View Medical Center, Los Angeles, April 5-6, 1995.
25. Grand Rounds lecture at Medical Oncology Conference in University of Rochester Cancer Center and Strong Memorial Hospital, Rochester, NY, March 9, 1995.
26. Grand Rounds lectures at The Oncology Institute of Loyola University Medical Center, Maywood, IL, October 10, 1993 and October 20, 1995.
27. Grand Rounds at University of Oklahoma, January 20-21, 1994.
28. Grand Rounds at City of Hope National Cancer Center, Duarte, CA, November 3, 1994.
29. Grand Rounds at The University of Arizona Cancer Center, Tucson, November 4, 1994.
30. Invited Lecture at Barnes Hospital, Washington University, St. Louis, MO, 1992.
31. Invited Lecture at Jewish Hospital, Washington University, St. Louis, MO, 1992.
32. Invited Participant, Monoclonal Antibody Conference at Memorial Sloan Kettering Cancer Institute, December, 1992.
33. Grand Rounds lecture at the Cancer Control Agency, University of British Columbia, Vancouver, BC, November 14, 1995.
34. Presentation to Zymogenetics Corporation Workshop on The Clinical Potential of Thrombopoietin, Seattle, February 4, 1994.
35. Presentation to NeoRx Corporation Scientific Advisory Board meeting, Seattle, April 27, 1995.
36. Presentation to Immunex Corporation Regional Meeting, San Diego, CA, September 23, 1994.
37. Presentation to Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, January 13, 1994.

Graduate Students Trained:

1. Francis Geissler M.D. Ph.D.
PhD.Thesis: Internalization and Degradation of Radioimmunoconjugates by malignant human lymphoid cells. (Ph.D. Degree conferred December, 1990)
2. Daming Shan M.D.
PhD Research: The effects of anti-B cell monoclonal antibodies on signal transduction, cell cycle progression, cell proliferation, and apoptosis in human B cell lymphomas.
(currently a 3rd year graduate student; Ph.D. expected in 1998-99.)
3. Aykut Bilge M.D. Ph.D.
PhD Thesis: Identification of the Intracellular Site of Ricin A Chain Translocation
(Ph.D. Degree December, 1995)
4. Deepa Venkataraman M.D.
PhD Research Project not yet finalized
(currently a 1st year graduate student; Ph.D. expected in 1999-2000).

Post-doctoral Fellows Trained:

Dr. Kenneth DeSantes
Project: Radiolabeled antibody targeting of the HER-2/neu oncoprotein (1988-1991).

SUMMARY OF EXPERIMENTAL FINDINGS

Aim 1: **To study the rates of endocytosis, intracellular routing, and metabolic degradation of radiolabeled monoclonal antibodies targeting tumor-associated antigens on human leukemia and lymphoma cells.**

Radiolabeled antibodies targeting differentiation antigens on human leukemia and lymphoma cells are internalized, routed via endosomes to lysosomes, degraded to small molecular weight metabolites, and then released from cells by exocytosis. Since the radiation dose absorbed by tumor cells is proportional to the mean residence time of RAbs in tumor sites, prolonging tumor retention of RAbs should generate not only improved external gamma camera images and improved radiation dosimetry, but also augmented radioimmunotherapeutic efficacy. To investigate this hypothesis, we studied the rates of endocytosis, intracellular degradation, and cell surface shedding of I-125-labeled monoclonal antibodies HD-37 (anti-CD19), B1 (anti-CD20), MB-1 (anti-CD37), BC8 (anti-CD45), and DA4-4 (anti-μ) using B lymphoma cells obtained by biopsying patients with Non-Hodgkin's lymphomas. The comparative behaviors of these radioimmunoconjugates were analyzed using cellular radioimmunoassays, ultrastructural autoradiography, SDS-PAGE analysis, and thin layer chromatography. I-125-BC8 (anti-CD45) was stably retained on the surface of lymphoma cells without appreciable internalization or shedding, whereas I-125-DA4-4 (anti-IgM) underwent rapid endocytosis and degradation (Fig. 1).

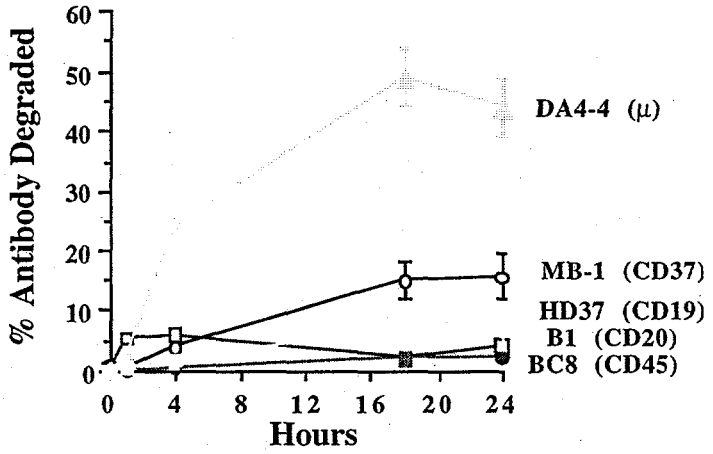

Figure 1: Degradation of I-125-labeled MoAbs by Human B Cell Lymphomas. Single cell suspensions prepared from 12 Non-Hodgkin's lymphoma biopsies were incubated with I-125 labeled antibodies HD37, B1, MB-1, BC8, or DA4-4 for 45 minutes at 4°C, washed and then incubated at 37°C for 0,1,4,18, or 24 hr before analyzing the supernatant fluid for the the presence of TCA soluble cpm. The data are presented as the % of initially bound cpm released in degraded, TCA-soluble form. Data for all patients were averaged and plotted with standard error bars.

I-125-B1 (anti-CD20) was not internalized or degraded by tumor cells.. Moderate rates of endocytosis, intracellular metabolism, and cell surface shedding were exhibited by I-125-HD37 (CD19) and I-125-MB-1 (CD37). Patients with diffuse, small cleaved cell lymphomas internalized and degraded antibodies more slowly than patients with other histologic subtypes. We believe that these kinetic differences may be important in the selection of MoAbs for immunotoxin and radioimmunoconjugate therapy of B cell malignancies. Our preclinical and clinical experiments, in combination with those of other workers, suggest that the ideal MoAb for I-131-radioimmunotherapy should exhibit a high degree of specificity for B cells, a slow antigen

association rate (to minimize the "binding site barrier" phenomenon), a slow dissociation rate (to maximize retention to tumor cells after binding), and a negligible endocytosis rate (to minimize intracellular degradation and release of low molecular weight I-131-catabolites). A manuscript describing these findings has been published. In addition, these laboratory observations have provided a basic understanding of radioimmunoconjugate behavior which has translated into successful Phase I and II clinical trials of therapy for relapsed NonHodgkin's lymphomas. The results of these studies suggest that antibodies which are slowly internalized and degraded (e.g. the anti-CD20 MAb B1 and the anti-CD45 MAb BC8) are best suited for radioimmunotherapy using Iodine-131, whereas rapidly internalized antibodies (e.g. the anti-CD19 antibody HD37 and the anti-CD22 antibody HD6) are most appropriate for applications using alternative radiolabeling methods (e.g. Yttrium-90, I-131-tyramine cellobiose, see below) or internalized toxins (e.g. ricin A chain).

Aim 2: **To examine the effects of lysosomotropic amines (e.g. chloroquine), carboxylic ionophores (e.g. liposomal monensin), and thioamides (e.g. propylthiouracil) on the retention of radiolabeled MoAbs by tumor cells *in vivo*.**

A variety of pharmacologic agents have been described which alter the intracellular routing of ligands or inhibit their catabolism inside cells. Lysosomotropic amines (NH_4Cl, amantadine, chloroquine), carboxylic ionophores (monensin), calcium channel blockers (verapamil), and lysosomal enzyme inhibitors (leupeptin) interfere with the delivery of ligands to lysosomes and/or inhibit lysosomal enzymes. The impressive efficacy of these agents in decreasing RAb degradation in lysosomal compartments and enhancing RAb retention by tumor cells *in vitro* has been well established, as shown in Fig. 2.

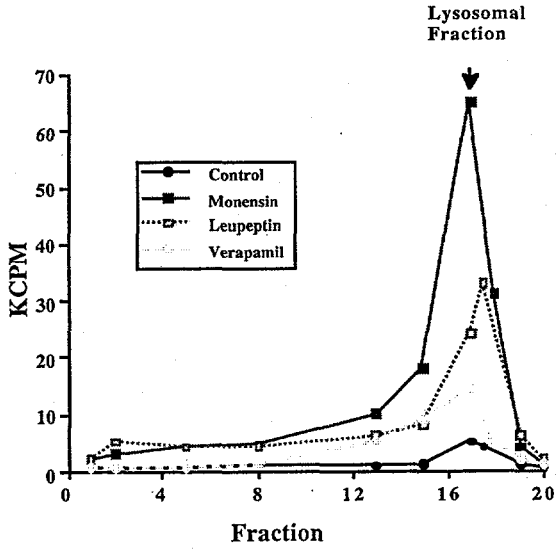

Figure 2: Enhanced retention of radioactivity in the lysosomal compartment of HPB-ALL cells pulse-labeled with I-125-MoAb 64.1 (anti-CD3) and incubated for 18 hours with 1μM monensin, 200 μM leupeptin, or 0.64 mM verapamil before lysing cells with a Dounce homogenizer and separating organelles on a 20% Percoll density gradient. The position of lysosomes on the gradient was determined by assaying fractions for ß-galactosidase activity by a fluorimetric assay.

Our attempts to extend these promising results with cell suspensions to mouse models have been hampered by toxicity including diarrhea, weight loss, and wasting which prevented achievement of maximally effective concentrations of the potentiators *in vivo*. Nevertheless, moderate enhancement of Rab retention by tumors was observed, with a 33% increase in dose to tumor and a 20% decrease in radiation dose to normal organs in animals treated with monensin (data not shown). We are continuing attempts to identify vehicles and schedules which will make the administration of higher concentrations of potentiators possible *in vivo*. One approach involves

the use of a liposomal formulation of monensin, which enhances its solubility and bioavailability (see Fig. 3).

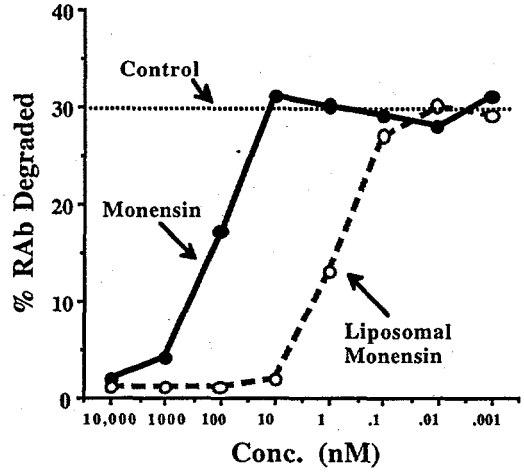

Figure 3: Liposomal monensin is 100 times more potent than aqueous monensin at inhibiting the degradation of I-125-DA4-4 (anti-IgM) antibodies by Ramos lymphoma cells as detected by measuring generation of TCA-soluble cpm in culture supernatants.

Aim 3: **To examine the impact of newer radioiodination techniques (e.g. tyramine cellobiose) on the metabolic degradation of radioiodinated antibodies, and on the radioimmunoscintigraphy and radioimmunotherapy of neoplasms.**

An alternative approach to enhance the retention of radioiodinated antibodies inside tumor cells employs novel new radioiodination methods using non-metabolizable carbohydrate adducts (e.g. tyramine cellobiose) which become trapped inside lysosomes after internalization. Cells appear to be unable to excrete these radioiodinated carbohydrate moieties from lysosomes even after proteolysis of the carrier antibodies. We have recently submitted a manuscript entitled "Tyramine cellobiose (TCB) antibody radioiodination: Pre-clinical studies for leukemia therapy" summarizing the synthesis and preliminary analysis of an anti-CD33 monoclonal antibody (P67) radioiodinated with either conventional chloramine T or novel tyramine cellobiose conjugation methods demonstrating the superiority of immunoconjugates synthesized with the TCB method compared with conventional chloramine T iodination. In vitro experiments show that only 38% of the radioiodine on P67 labeled by chloramine T remained associated with cells after 24 hours of incubation while 72% of the TCB labeled radioiodine remained cell-associated (Fig. 3).

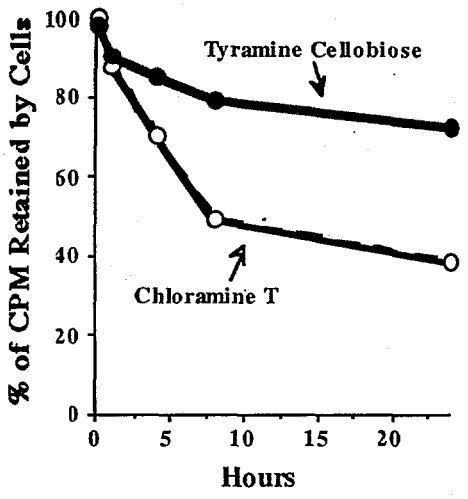

Fig. 6: Enhanced retention of ^{125}I-MoAb P67-6 by HEL cells after conjugation by the tyramine cellobiose method compared with the chloramine T method (as determined by rate of accumulation of TCA soluble cpm in culture supernatants).

Biodistribution experiments in mouse tumor models co-injected with I-125-chloramine T or tyramine cellobiose (TCB) labeled P67 also showed an increased tumor concentration and retention of the TCB conjugates with a 4.5 fold greater retention as assessed by an area under the curve (% ID/gram vs. time analysis). Concentrations of radioiodine in other tissues were comparable for both TCB and CT labels except in the liver where TCB was twice that of CT (Fig. 4).

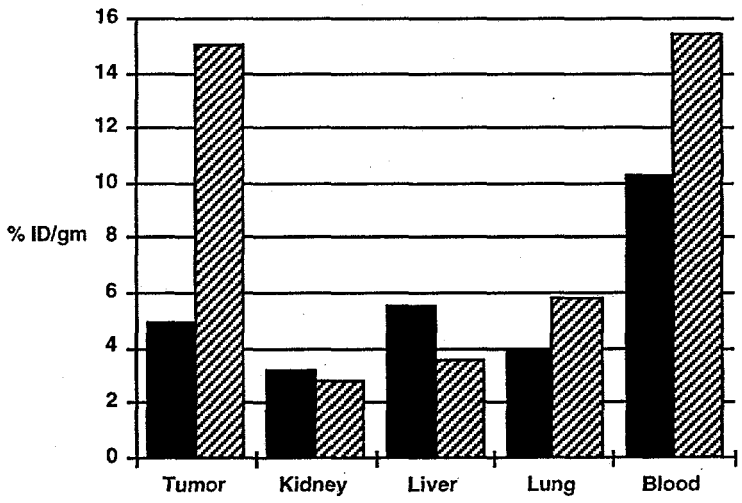

Figure 4: Comparative Biodistributions of Chloramine T (in black) and Tyramine Cellobiose (hatched) labeled I-125-P67 (anti-CD33) antibody in nude mice bearing Human Erythroleukemia (HEL) tumor xenografts 48 hours after antibody injection.

Aim 4: **To compare the endocytosis, intracellular routing, and degradation of radioimmunoconjugates prepared with different radionuclides. (Iodine-131, Indium-111, Yttrium-90).**

The selection of therapeutic radionuclides for clinical trials remains controversial. We compared the internalization, lysosomal targeting, metabolism, and cellular retention of radiolabeled, humanized monoclonal antibodies targeting the CD33 antigen (MoAb hP67) on leukemia cell lines (HEL, HL-60) , and of anti-carcinoma antibodies (MoAbs hCTM01 and hA33) targeting breast cancer and colorectal carcinoma cell lines (MCF7 and Colo 205, respectively). Each antibody was labeled with I-125 (by the IodoGen method) and with In-111 and Y-90 using macrocyclic chelation technology (using the 9N3 and 12N4 proprietary chelates provided by CellTech®). Targeted tumor cells were analyzed for retention and metabolism of radioimmunoconjugates using cellular radioimmunoassays, Percoll gradient fractionation of cell organelles, SDS-PAGE, and thin layer chromatography of cell lysates and culture supernatants. Our results suggest that antibodies are routed to lysosomes after endocytosis where they are proteolytically degraded. I-125-monoiodotyrosine is rapidly excreted from cells after lysosomal catabolism of antibodies radioiodinated by conventional methods, whereas small molecular weight In-111 and Y-90 catabolites remain trapped in lysosomes. As a consequence of the differential disposition of small molecular weight catabolites, In-111 and Y-90 conjugates displayed superior retention of radioactivity compared with I-125 conjugates when tumor cells were targeted using rapidly internalizing antibody-antigen systems (e.g. hP67 with HEL cells, hCTM01 with MCF7 cells [Fig. 5]).

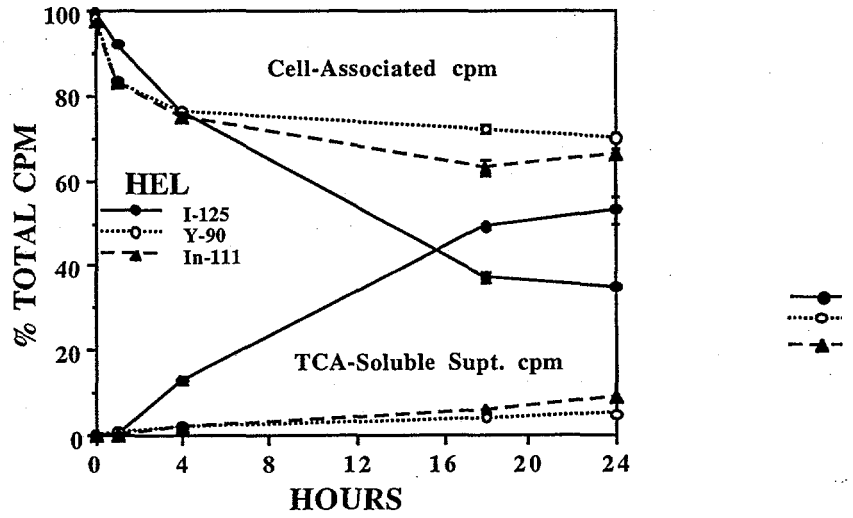

Figure 5. Cell-associated and TCA-soluble supernatant radioactivity in cultures of HEL cells incubated for 0,1,4,18, or 24 hours after surface labeling with I-125-hP67 (filled circles), In-111-hP67 (solid triangles), or Y-90-hP67 (open circles). 10^6 HEL cells were incubated with 150 ng of trace-radiolabeled hP67 at 4°C, washed, placed in fresh culture medium and incubated at 37°C for 0, 1, 2, 4, 18 and 24 hours before quanitifying the amounts of cell-associated (top three curves) and TCA-soluble, supernatant radioactivity (bottom three curves).

However, when tumor cells were targeted using antibody-antigen systems exhibiting slow rates of endocytosis (e.g. hP67 on HL-60 cells, hA33 on Colo 205 cells), little difference in cellular retention of radioactivity was observed regardless of whether I-125, In-111, or Y-90 was employed (Fig. 6).

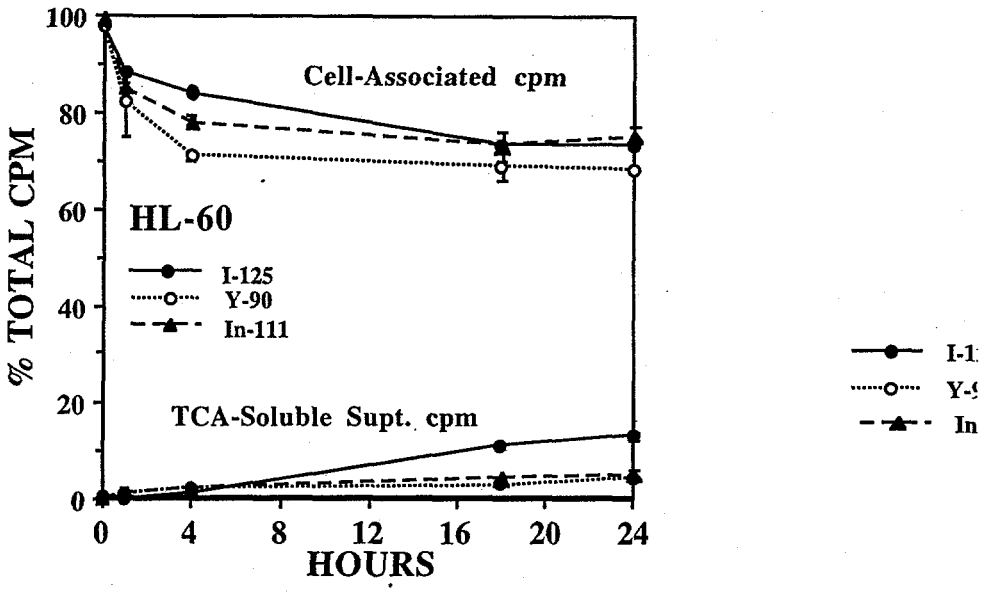

Figure 7. Cell-associated and TCA-soluble supernatant radioactivity in cultures of HL-60 Cells incubated for 0,1,4,18, or 24 hours after surface labeling with I-125-hP67 (filled circles), In-111-hP67 (solid triangles), or Y-90-hP67 (open circles) as described in the legend to Figure 1. Mean cpm bound per 10^6 cells at time 0 were 44,490 ± 228 for I-125 , 12, 145 ± 1928 for In-111, and 7333 ± 284 for Y-90.

The results of these experiments are discussed fully in a manuscript entitled "Comparative metabolism and retention of Iodine-125, Yttrium-90, and Indium-111 radioimmunoconjugates by cancer cells" which has recently been submitted for publication.

Aim 5: **To examine the utility of radioimmunoconjugates targeting oncogene products for the radioimmunotherapy and radioimmunoscintigraphy of cancer.**

In an effort to extend our clinical successes with hematologic malignancies to solid tumors, we studied the utility of treating poor prognosis breast and ovarian carcinomas with RAbs targeting the Her2/neu (c-erb B2) oncogene (in collaboration with Dr. Dennis Slamon, UCLA). Cellular radioimmunoassays demonstrated that breast and ovarian cancer cell lines (SKBR3, SKOV3) and fibroblasts transfected with the HER2/neu gene internalize and degrade anti-HER2/neu antibodies in a manner identical to that described above for our lymphoma studies (126). Experiments in a nude mouse xenograft system documented specific localization of injected ^{131}I-4D5 to Her2/neu bearing tumors with tumor to non-tumor ratios of 5-10 to 1. Gamma camera imaging demonstrated excellent tumor localization. Pilot studies have demonstrated the radioimmunotherapeutic efficacy of this approach (Fig. 8), though all tumors have eventually regrown at the three dose schedules tried so far. Unfortunately, insufficient funds were available to continue this project, and since 1993 we have focussed the available resources exclusively on our leukemia and lymphoma studies.

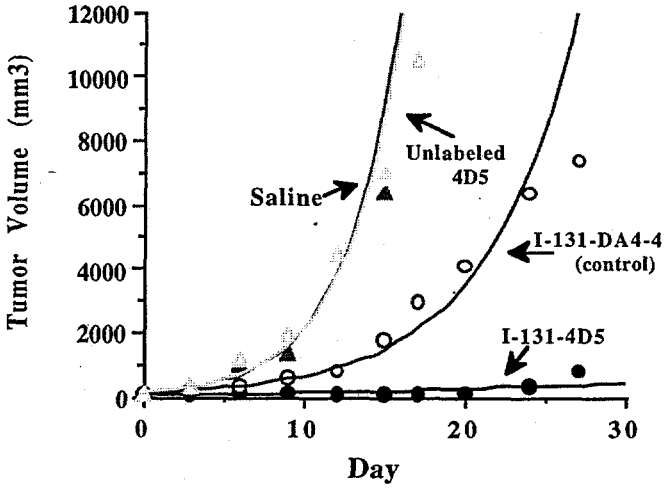

Fig. 8: Inhibition of growth of Her2/neu expressing tumor xenografts in beige/nude mice injected on Day 0 with a single dose of anti-Her2/neu RAb I-131-4D5 (400 μCi/45 μg), I-131-DA4-4 (400 μCi/45 μg of nonbinding MoAb), 45 μg unradiolabeled MoAb 4D5, or saline.

Other Preliminary Data:

Although we believe that most of the clinical responses we have observed on our Phase I & II clinical trials using RAbs have resulted from the antibody-directed targeting of ionizing radiation (in the form of I-131) to tumor cells, an increasing body of *in vitro*, mouse xenograft and clinical data strongly suggest that clinically important tumor regressions can be achieved using unmodified anti-CD19 and CD20 MoAbs. These responses appear to be partially due to recruitment of host effector cells mediating antibody-dependent cellular cytotoxicity, and partially due to perturbation of lymphoid signal transduction pathways mediating cell cycle arrest and apoptosis. To achieve maximal cytotoxic efficacy, we plan to dissect the transduction pathways involved and determine the optimal conditions for harnessing these cytostatic and cytotoxic effects.

As a preliminary investigation of this approach we have tested the ability of murine anti-CD20 antibodies to inhibit DNA synthesis and cell proliferation of malignant B cell lymphoma lines and to induce cell cycle arrest and apoptosis. We have found that the 1F5 anti-CD20 antibody is a potent inhibitor of DNA synthesis of the malignant Ramos Burkitt's lymphoma cell line (Fig. 9).

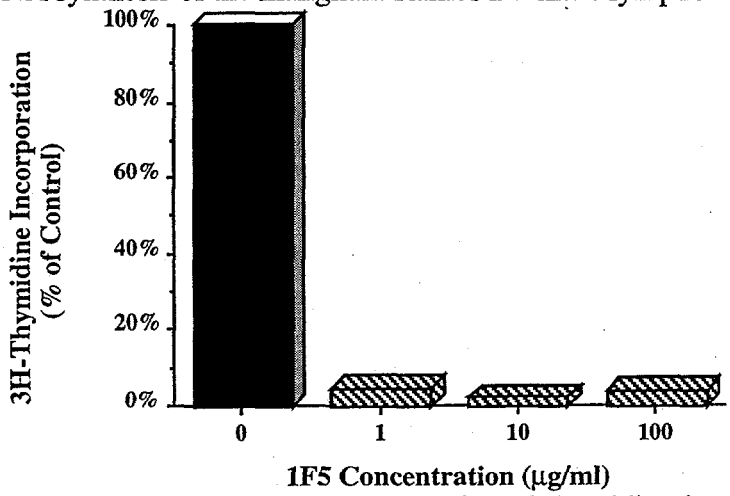

Fig. 9 Inhibition of DNA synthesis (as measured by tritiated thymidine incorporation) in Ramos cells by the anti-CD20 MoAb 1F5. A control antibody 64.1 (anti-CD3) had no effect on tritiated thymidine incorporation (not shown).

Our data also suggest that 1F5 anti-CD20 antibody enhances tyrosine phosphorylation of multiple intracellular substrates including PLCγ1 (data not shown). These transduction events appear to culminate in fragmentation of DNA in targeted B cells (Fig. 10).

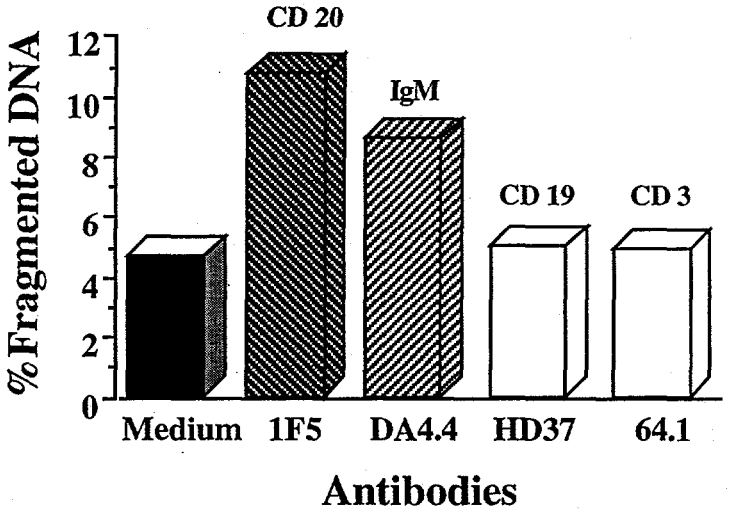

Fig. 10 Incubation of malignant human B cells (Ramos) with anti-CD20 (1F5) or anti-IgM antibodies (DA4-4) increases fragmentation of DNA as assessed by propidium iodide assay. Anti-CD19 (HD37) and anti-CD3 (64.1) antibodies did not have a significant effect on DNA fragmentation in these experiments.

Unexpectedly, the effect of anti-CD20 antibodies on inhibition of DNA synthesis and fragmentation of DNA was highly sentitive to cell density, with maximal effects exerted at low cell density for both phenomena (Figs 11 & 12). The effect of higher cell density could not be compensated by adding more 1F5 antibody, since the inhibitory effects of 1F5 were abrogated by higher cell density even when 100-fold increases of 1F5 antibody were used.

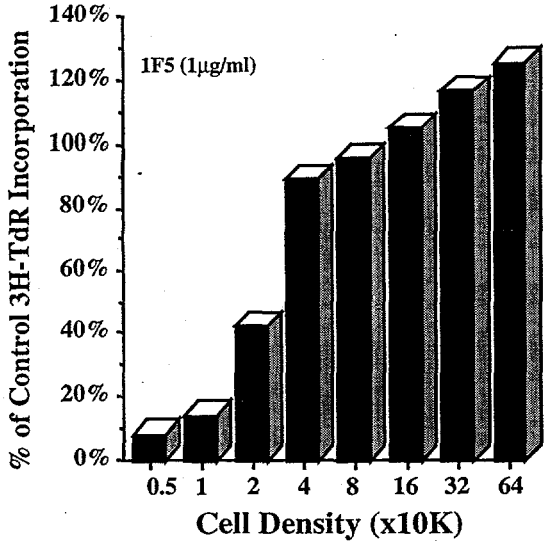

Fig. 11: Effect of cell density on the anti-proliferative effect of anti-CD20 antibody 1F5

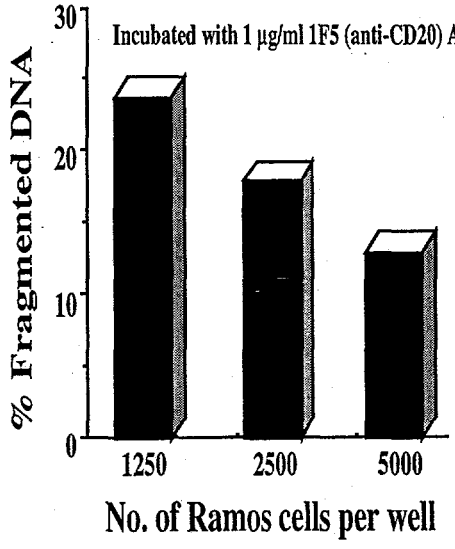

Fig 12: Effect of cell density on the % of fragmented DNA detectable by propidium iodide

(1μg/ml) incubated with Ramos cells (assessed by incorporation of tritiated thymidine in wells incubated with 1F5). Data are expressed as % of cpm incorporated in wells containing the same number of cells, but incubated without 1F5). Density expressed as number of cells in 200 μl wells.

staining in cultures of Ramos cells incubated with 1 μg/ml anti-CD20 antibody 1F5. Volume of wells = 200 μl.

Analysis of 1F5-coated Ramos cell lysates by agarose gel electrophoresis demonstrated DNA segmentation ladders typical of apoptotic cell death in 1F5-treated cells, particularly in cultures where anti-CD20 antibodies were cross-linked with goat-anti-mouse Ig antibodies. We anticipate that knowledge of the molecular pathways involved in these processes will allow them to be exploited clinically to maximize lymphoma cell killing.

As a preliminary exploration of the possible utility of combining chemotherapy and radioimmunotherapy, we have administered I-131-anti-CD20 B1 antibody (at doses delivering 20 Gy to normal organs) in conjunction with cyclophosphamide (100 mg/kg) with (five patients) or without etoposide (four patients, 60 mg/kg) and autologous bone marrow transplantation (Fig. 13).

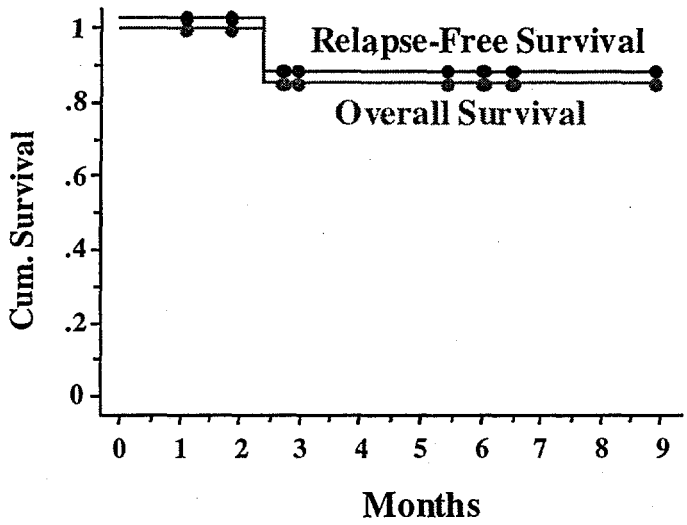

Fig. 13: Preliminary analysis of overall and relapse free survival of patients on an ongoing clinical trial of I-131-B1 antibody (2.5 mg/kg, ~600 mCi), etoposide (100 mg/kg), and cyclophosphamide (100 mg/kg) followed by autologous bone marrow transplantation (Kaplan-Meier survival analysis).

None of the patients has experienced progressive lymphoma so far, but follow-up is very short (1-9 months), and all patients sustained significant toxicity include myeloablation requiring bone marrow rescue, multiple transfusions, and fever requiring antibiotics. In addition, the four patients treated with etoposide developed painful mucositis, one patient sustained serious but reversible veno-occlusive disease of the liver, and another developed fatal disseminated Varicella zoster infection. Although these toxicities are justifiable in patients with an incurable malignancy, it would be desirable to identify a less toxic regimen which still induces long term remissions.

The selection of cyclophosphamide and etoposide as combination therapy for I-131-B1 was based on their inclusion in the most common bone marrow transplant regimen used in the United States for relapsed Non-Hodgkin's lymphomas, namely external beam total body irradiation, cyclophosphamide and etoposide. Nevertheless, it seems likely that a superior combination with a better therapeutic index (efficacy vs toxicity) might be identified for use with I-131-B1 . Given the

multitude of potential agents for testing (see Background) and the time and logistic difficulties of testing multiple combinations in patients, we propose a systematic preclinical analysis of the leading agents *in vitro* and in mouse models, which can be translated in our next patient trials.