Evaluation of Combined Modality Radioimmunotherapy for Treatment of Mammary Adenocarcinoma using in vivo Bioluminescence Imaging

HYPOTHESIS:
Radioimmunotherapy is a viable option for targeted irradiation of chemotherapy-resistant tumor cells.

PROJECT DESCRIPTION (Include design, methodology, data collection, techniques, data analysis to be employed and evaluation and interpretation methodology)

This study will use the MCF-7-lucF5 human breast cancer cell line (Caliper Life Sciences, Hopkinton, MA). This cell line is derived from the MCF-7 human adenocarcinoma cell line and transfected with the North American Firefly Luciferase gene. The cell line is estrogen dependent and will serve as a xenograft model of estrogen dependent breast cancer.

The RIT agent used for this study will target the P7 antigen known to be expressed on multi-drug resistant cancer cells (10-12). The Mab, anti-P7, which recognizes the P7 antigen, will be conjugated with $^{131}$I.

A total of 20 female NIH III homozygous (NIH-Long-Eared Foxn1nuBtkxid) nu/nu beige tumor-bearing mice 4-6 weeks old will be used for this study. Since the cancer cell lines used are estrogen dependent, the mice will first be implanted with estrogen pellets (17β-Estradiol, 0.36 mg/pellet, 60 day release). Two days following estrogen implantation, the mice will be inoculated with $7 \times 10^6$ cells of MCF-7-lucF5 into the abdominal mammary fat pad. One week following inoculation, luciferin will be injected intraperitoneally. Mice will be anesthetized and imaged using the in vivo imaging system (Xenogen IVIS 200 Series, Caliper Life Sciences, Hopkinton, MA) of UMDNJ Core Facilities to confirm the presence of tumors. Following confirmation of tumor presence, mice will be randomly assigned to four groups of five mice. Each group will receive a different treatment to be administered intraperitoneally:

- **Group 1- Control:** Treated with 10% ethanol in saline
- **Group 2- Chemotherapy:** Treated with doxorubicin and paclitaxel
- **Group 3- Radioimmunotherapy:** Treated with anti-P7 antibody conjugated with Iodine-131 ($^{131}$I anti-P7)
- **Group 4- Chemotherapy + Radioimmunotherapy:** Treated with a cocktail of doxorubicin and paclitaxel with ($^{131}$I) anti-P7

Mice will be injected with luciferin and imaged weekly using both the IVIS as well as planar nuclear medicine imaging (Anzai ex-Scope AN Portable Gamma Camera, Anzai Medical Company, Tokyo, Japan) for a total of 8 weeks following administration of treatment. Images from both modalities used will be superimposed using standard image fusion techniques. Assistance with nuclear medicine imaging and image fusion will be provided by Dr. Lionel Zuckier. Following the 8 week observation period, the mice will be euthanized and tumors will be excised. Expression of the P7 antigen on the excised tumor cells will be evaluated using both Western immunoblot analysis and immunohistochemical techniques.
Summer Student Research Program
Project Description


IS THIS PROJECT SUPPORTED BY EXTRAMURAL FUNDS?

Yes ☒ or No ☐

*IF YES, PLEASE SUPPLY THE GRANTING AGENCY'S NAME*

NIH/NCI

THIS PROJECT IS: ☒Clinical ☐Laboratory ☐Behavioral ☐Other

THIS PROJECT EMPLOYS RADIOISOTOPES ☒

THIS PROJECT INVOLVES THE USE OF ANIMALS ☒

PENDING ☒ APPROVED ☐ IACUC PROTOCOL #

THIS PROJECT INVOLVES THE USE OF HUMAN SUBJECTS ☐

PENDING ☐ APPROVED ☐ IRB PROTOCOL # M
WHAT WILL THE STUDENT LEARN FROM THIS EXPERIENCE?

The student will:
1. Receive training in radiation safety, research animal safety, and laboratory safety.
2. Radiolabel the anti-P7 with I-131 with guidance from nuclear medicine physician, Lionel Zuckier MD, who has extensive antibody labeling experience.
3. Inoculate luciferin expressing tumor cells into the fat pad of female nude mice. Implant estrogen pellets. The student will be trained in these techniques by animal care staff.
4. Anesthetize mice and follow tumor growth with the IVIS 200 imaging system at numerous times after inoculation.
5. Inject radiolabeled chemotherapy drugs and I-131 labeled anti-P7 with the assistance of John Akudugu PhD, a post-doctoral fellow in my laboratory.
6. Obtain planar gamma camera images of the I-131 injected animals and merge resulting image with IVIS 200 images. Dr. Zuckier will provide guidance on these steps.
7. Analyze all data to determine added anti-tumor effect of the radiolabeled anti-P7.
8. With the assistance of Dr. Edouard Azzam's post-doctoral fellows, perform western blot analyses on control and treated tumor tissues. Cut sections of tumor tissues for immunohistochemistry analysis by imaging core facility.