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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The proposed research will develop a new way to treat prostate cancer. We have discovered prostate cancer antigens that can be induced by radiation therapy. Because radiotherapy is a primary mode of treatment of both localized prostate cancer and metastatic prostate cancer. These antigens are expressed during therapy. We have developed antibodies to two of the lead radiation inducible antigens, TIP-1 and GRP-78. The proposed research will study how effective these antibodies are at activating an immune response against prostate cancer.

The study also proposes to study these antibodies for planned future clinical trials. We will label the antibodies with radiotracers that can be imaged by PET scans. We will image mouse models of prostate cancer to study the cancer specific binding of the antibodies. The goal of this research is to submit the imaging data to the FDA for our future planned Investigational New Drug application. This research will develop a new paradigm in treatment of prostate cancer by use of therapeutic antibodies targeting inducible antigens in prostate cancer.

15. SUBJECT TERMS
None Listed

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INTRODUCTION

The overall goal of the proposed research is to test the hypothesis that therapeutic antibodies can be guided specifically to radiation-inducible neoantigens that are overexpressed in prostate cancer. The limitation of this approach is the paucity of cancer specific antigens that bind therapeutic antibodies. To address this limitation, we studied inducible neoantigens on cancer. This is a new paradigm in therapeutic antibody development**.** Inducible neoantigens markedly expand the number of therapeutic targets for antibody development. We prioritized inducible proteins that are over-expressed in irradiated cancer and subsequently activate immune effector cells. Glucose Regulated Protein-78 (GRP78), and Tax interacting protein-1 (TIP1) are overexpressed in cancer and participate in the stress response that occurs in irradiated cancers. Cancer cells show an exaggerated response with markedly increased expression of TIP1, and GRP78 on their surface as compared to little or no expression in irradiated normal tissues. GRP78 and TIP1 are among the lead molecular targets for therapeutic antibody development because they are induced with low doses of radiation, and they activate immune effector cells.

The objective of this research was to address a critical barrier and develop a new paradigm in cancer treatment. Development of anti-cancer antibodies is limited by the paucity of antigens that are specifically over-expressed in cancer resulting in too few molecular targets and small percentages of patients who can be treated with therapeutic antibodies. We have recently established a platform technology to develop antibodies to radiation-inducible neoantigens, which occurs in nearly all cancers. Ionizing radiation induces a stress response resulting from DNA strand breaks and activation of ATM [1, 2]. Tax interacting protein-1 (TIP1) are induced at high expression levels on the cell surface of cancers [3-5]. We hypothesize that therapeutic antibodies targeting radiation inducible antigens can be used to activate the immune response to cancer. In particular, we will study the use of monoclonal antibodies targeting radiation inducible antigens and determine the biological impact of antibody dependent cell mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP). This research will allow us to characterize antigens and antibodies intended for clinical trials in patients with NSCLC. Our preliminary data has identified TIP1 as candidate radiation inducible neoantigens that are expressed on the surface of irradiated cancer cells for prolonged times during fractionated irradiation. Expression of TIP1 is specific to cancer and provides a new class of antigens for the development of therapeutic antibodies to irradiated prostate cancer. TIP1 [6] [4, 7] is a membrane associated protein that is over-expressed in cancer [3-5]. We recently found that TIP1 undergoes radiation induced translocation to the cell surface [3-5]. TIP1 is expressed specifically on cancer cells for several days after irradiation. Whole animal imaging and immunohistochemical staining studies indicate that anti-TIP1 monoclonal antibody, 2C6F3, binds specifically to irradiated cancers in mouse models. TIP1 has been prioritized among inducible antigens because it is specifically expressed on irradiated cancer cells, and remain displayed on the cell surface and accessible to antibody binding for prolonged periods of time (9 days). Moreover, TIP1 is inducible in nearly all mouse models of cancer resulting in opsonization and activation of ADCC and ADCP. Antibodies that we developed and prioritized include 2C6F3, which is a mouse monoclonal IgG2b antibody recognizing TIP1. 2C6F3 binds specifically to irradiated cancers in vivo.

KEY WORDS:

Glucose Regulated Protein-78 (GRP78)
Tax interacting protein-1 (TIP1)
IgG
antibody dependent cell mediated cytotoxicity (ADCC)
antibody-dependent cell-mediated phagocytosis (ADCP).
ScFv antibodies

Overall Project Summary

Subtask 1.1
Binding of antibodies to irradiated cancer cells.
Mice were inoculated with human TIP1 protein and B lymphocytes were harvested from the spleens. B cells were fused to myeloma cells and hybridomas were cloned and selected based on antibody binding to the surface of irradiated prostate cancer cells.
We studied human prostate cancer cell lines PC3 and DU145. We first studied antibody binding to irradiated cancer cell lines. We found that the lead anti-GRP 78 antibody binding to cancer cell lines was monoclonal antibody 4A6O9. Flow cytometry analysis of antibody binding to the surface of cancer cells was performed in the following manner: cancer cell lines were grown in culture and irradiated with 3Gy. Cells were then scraped with EDTA to create single cell suspensions. Following irradiation, the cells were incubated with all of our lead mouse monoclonal antibodies. We found that clone 4A6O9 showed the greatest amount of binding to the surface of irradiated cancer cells (Figure 1). In comparison, the commercially available polyclonal antibody to GRP 78 is indicated as DD. Of the TIP-1 monoclonal antibodies, we found that 2C6F3 showed the greatest level of binding (Figure 1B).

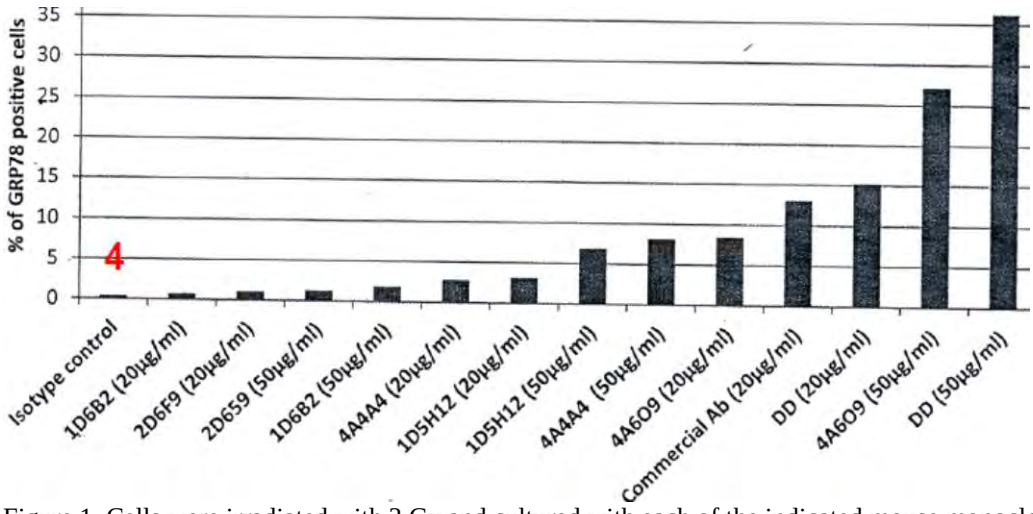

Figure 1. Cells were irradiated with 3 Gy and cultured with each of the indicated mouse monoclonal antibodies. Bar graph showing the percentage of cells staining positive with each of the antiGRP-78 antibodies is shown. 4A6O9 showed the greatest level of binding to irradiated cancer cells. The commercial antibody (PD) is a polyclonal antibody that was used as a positive control.

The next prioritized lead ScFv antibodies binding to each of these antigens. We found that the C14 ScFv antibody bound with greatest specificity to PC3 tumors. Figure 2 shows anti-GRP78 ScFv antibody binding to PC3 tumors following irradiation with 3 Gy. The histogram shows the labeled antibody binding within the tumor region of interest. PC3 tumors were implanted into nude mice and irradiated with 3Gy. The antibodies were then labeled with ALX750 fluorescent dye and mice were imaged by near infrared imaging. This research indicated that the lead antibody to GRP78 was the C14 ScFv antibody (Figure 2). The lead ScFv binding to TIP-1 was TE11. Figure 3 shows histogram of the tumor as a region of interest. This was acquired from near infrared imaging of the TE11 anti- TIP-1 antibody binding to irradiated cancers in nude mice.

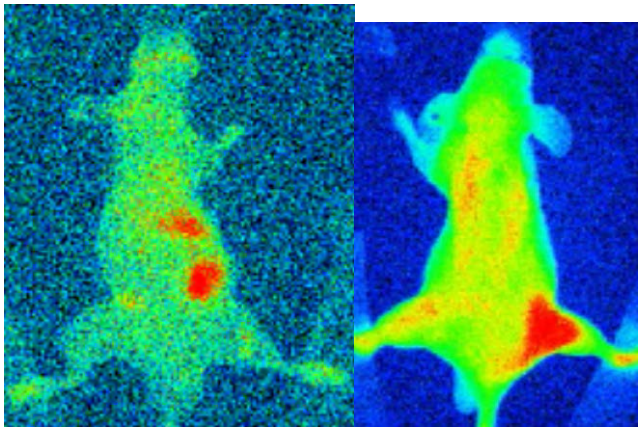

Figure 2. Near infrared imaging of mice bearing X irradiated tumors. Left panel shows imaging of the control SCFE antibody. Right panel shows imaging of the C 14 SCFE antibody to GRP 78.

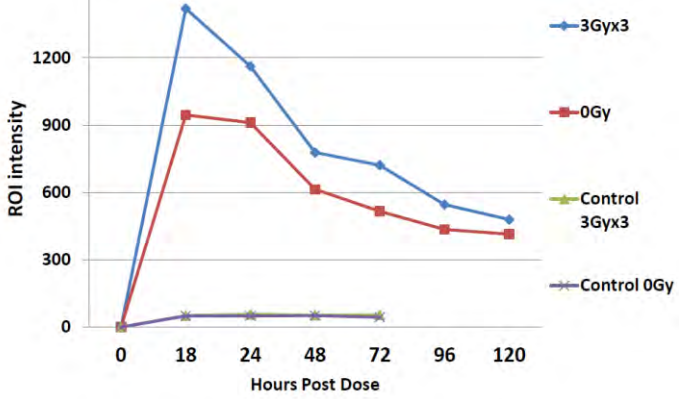

Figure 3. Histogram showing the photon emission from tumors treated with TE 11 antibody labeled with ALX 750.

To determine the efficacy of antibodies combined with irradiation, we first studied the effects of the antibodies added to cells in culture following irradiation. Cells were treated with radiation and antibody was added immediately after treatment. Cell survival was measured by colony formation and cell count. The anti-GRP78 antibody enhanced the therapeutic efficacy of irradiation as shown in (Figure 4). The anti-TIP-1 antibody also showed enhanced efficacy of radiation on cancer cells in culture (Figure 5).

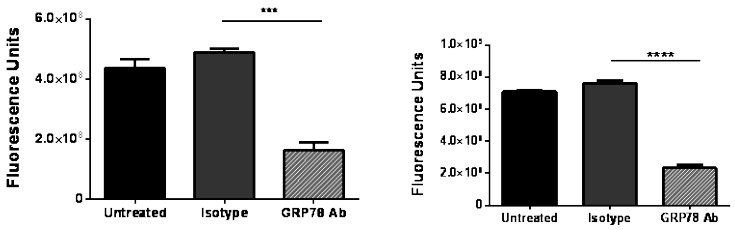

Figure 4. Bar graph showing MTT assay of cancer cells treated with antibodies and radiation. The anti GRP-78 antibody and enhanced cytotoxicity is compared to isotype control antibody (***P<0.001).

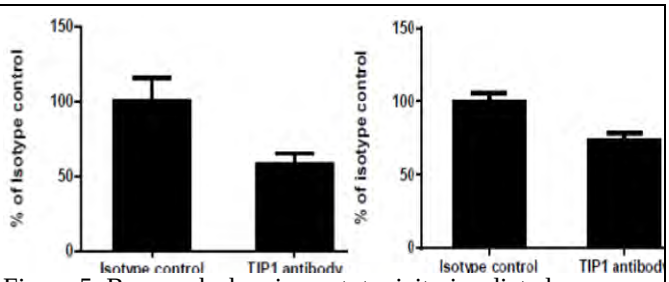

Figure 5. Bar graph showing cytotoxicity irradiated cancer cells treated with the anti TIP-1 antibody following irradiation. The anti TIP 1 antibody reduced cell survival to 50% as compared to isotype control antibody.

Major Task 2

We next studied cancer specific binding of radiolabeled antibodies in mouse models of prostate cancer. Antibodies were conjugated to DTPA. We labeled the lead monoclonal antibody 2C6F3 with In-111. Mice were then imaged with SPECT. Figure 6 shows SPECT imaging of radiolabeled antibody binding to irradiated tumors in the hind limb following X irradiation, but not in untreated control (SHAM). We also studied near infrared imaging of 2C6F3 in mice bearing irradiated tumors in the hind limb. Shown are NIR images of the 2C6F3 antibody on the right as compared to the control antibody on the left.

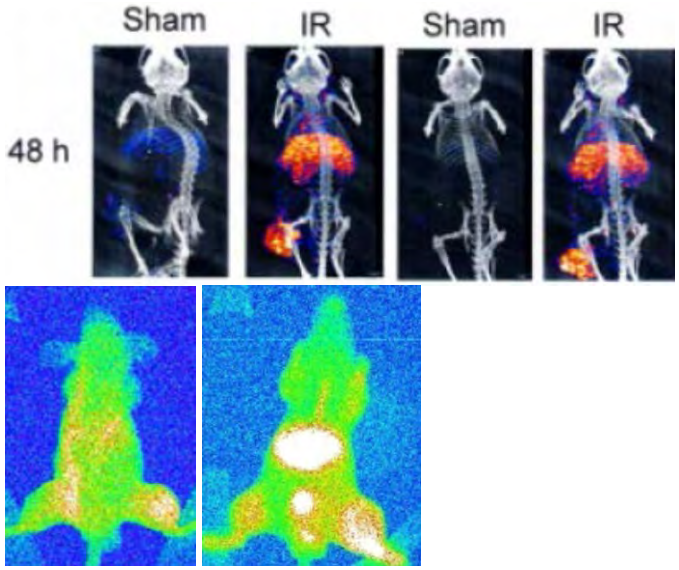

Figure 6. Upper panels show SPECT imaging of In-111 labeled antibody in mice bearing tumors. SHAM irradiated controls received 0 gray and IR treated tumors received 3 gray. Images were obtained 72 hours following administration of radiolabeled antibody. Lower panels show near infrared imaging of control SCFE antibody (left panel) and 2C6F3 labeled antibody in the right panel.

Major Task 3

To study the activation of immune effector cells, we determined the activation of NK cells when co-cultured with irradiated cancer cells. 2C6F3 antibodies were added to cell culture following irradiation. NK cells were then co-cultured with these live cells following antibody binding. Figure 7 shows NK cell activation. This essay measures the release of LDH into the medium following NK cell mediated lysis of cancer cells. These data demonstrate that lead antibody 2C6F3 is capable of activating NK cell mediated antibody dependent cytotoxicity when NK cells are added to prostate cancer cells treated with 3 Gy and 2C6F3.

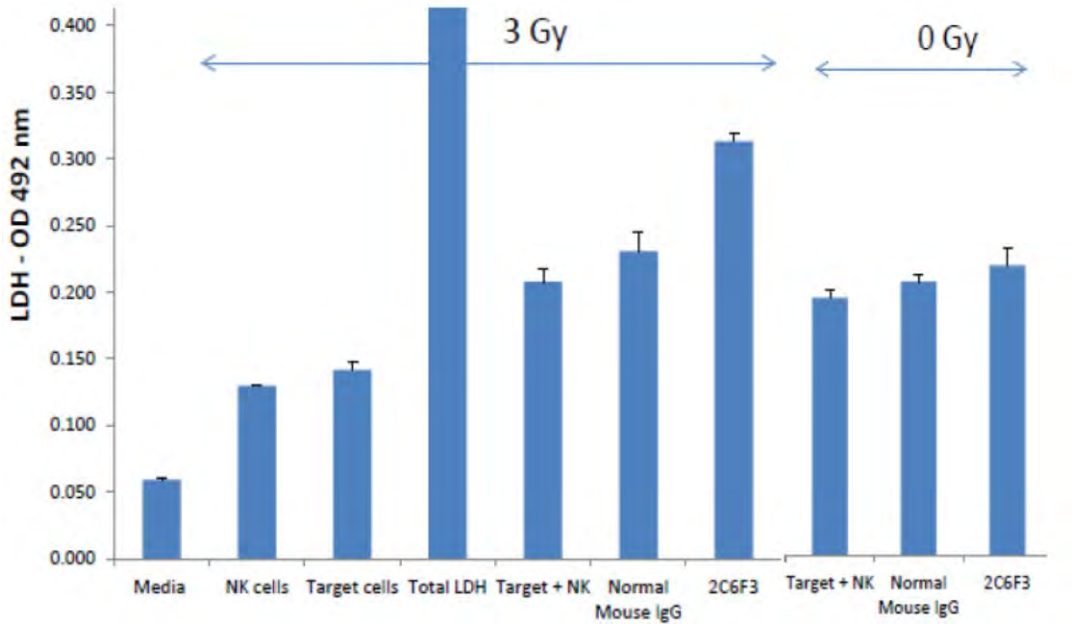

Figure 7. Antibody dependent cell mediated cytotoxicity: NK cell mediated cytolysis. 2C6F3 was added to cancer cell culture with or without irradiation (3Gy vs 0 Gy). Cells were then washed and co-cultured with NK cells. We quantified the amount of LDH released during cytolysis of cancer cells. Shown are the mean and standard error from three experiments with each of the indicated conditions. Normal mouse IgG was compared to 2C6F3.

Key Research Accomplishments:

We found that antibodies to TIP-1 bind specifically to prostate cancer cells following irradiation. We also found that TIP-1 antibodies binds specifically to irradiated prostate cancer tumors in nude mice. We've radiolabeled antibodies and found that we could maintain cancer specific binding in prostate cancer models in mice. We next studied the ability of our lead antibody 2C6F3 to activate immune response in irradiated cancer cells. This showed activation of NK cells when co-cultured with prostate cancer cells with antibody.

CONCLUSIONS:

Atibodies to TIP-1 and GRP78 bind specifically to prostate cancer cells following irradiation. Anti-TIP-1 and anti-GRP78 antibodies also bind specifically to irradiated prostate cancer tumors in nude mice. Radiolabeled antibodies maintain cancer specific binding in prostate cancer models in mice. Our lead antibody 2C6F3 to TIP1 activates immune effector cells on irradiated prostate cancer cells. NK cells were activated when co-cultured with cancer cells with antibody.

In conclusion, radiation can be used to activate new antigens in prostate cancer. Because this is a primary treatment modality for prostate cancer, we plan to conduct clinical trials of antibodies to TIP-1 in patients receiving radiotherapy for radiation.

Publications, Abstracts, and Presentations

none

Inventions, Patents and Licenses

none

Reportable Outcomes

Not applicable

Other Achievements

none

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