Lankenau Institute for Medical Research

Annual Progress Report: 2012 Formula Grant

Reporting Period

July 1, 2013 – June 30, 2014

Formula Grant Overview

The Lankenau Institute for Medical Research received $115,879 in formula funds for the grant award period January 1, 2013 through December 31, 2014. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Fluorodeoxyglucose and HEDS to Improve Human Colon Cancer Cells Response to Radiation – There is a great interest in the inhibitors of glucose metabolism since glycolysis and oxidative pentose phosphate cycle (OPPC), which require glucose, play a major role in the survival and radiation response of tumor. Fluorodeoxyglucose (FDG), believed to be an inhibitor of glucose metabolism, is used for the positron emission tomography (PET) of tumors. Although FDG has been used for PET, it is not known whether FDG is a potent inhibitor of OPPC and thiol redox homeostasis, and improves the radiation response of tumor cells. We will determine the combinatorial therapy of FDG and hydroxyethyl disulfide, which is known to induce oxidative stress in OPPC deficient cells, in improving the radiation response of human colon cancer cells.

Duration of Project

1/1/2013 – 6/30/2013

Summary of Research Completed

This project ended during a prior state fiscal year. For additional information, please refer to the Commonwealth Universal Research Enhancement C.U.R.E. Annual Reports on the Department's Tobacco Settlement/Act 77 web page at http://www.health.state.pa.us/cure.

Research Project 2: Project Title and Purpose

Preclinical Evaluation of a Novel Experimental Therapy for Multiple Myeloma – This study will employ an established preclinical model of multiple myeloma (MM), an aggressive blood cancer composed of B immune cells, to evaluate the safety, pharmacology and efficacy of a novel experimental therapy developed by scientists at the Lankenau Institute. The therapy is a monoclonal antibody that blocks a signaling molecule abundantly produced by MM cells and hypothesized to be required for their uncontrolled growth on the basis of genetic studies of its

role in cancer and immune disease. Positive results from the work would provide needed justification for its clinical evaluation in MM patients.

Anticipated Duration of Project

10/17/2013 - 12/31/2014

Project Overview

Core Research Objective**.** Evaluate the preclinical safety, pharmacokinetics and efficacy of an experimental monoclonal antibody (mAb) therapy in an established model of multiple myeloma, a lethal cancer composed of malignant plasma B cells that accumulate in the bone marrow.

Specific Aims
Aim 1. Prepare and purify mAb for testing and conduct quality controls of the preparation.
Aim 2. Test the effect of mAb treatment on the growth and survival of MM cells *in vitro*.
Aim 3. Explore the safety and pharmacology of mAb in wild-type and MM-bearing mice.
Aim 4. Evaluate the preclinical efficacy of mAb in MM-bearing mice

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Expected Research Outcomes and Benefits

This research could lead to the development of a more effective therapy for multiple myeloma (MM), an aggressive and lethal blood cancer with an incidence of approximately 20,000 new cases per year in the U.S. Overall, MM is a common blood cancer, representing 1% of all cancers in the U.S. but 2% of all cancers in African-Americans, illustrating a cancer disparity in this population. While some progress has been made in treatment, specifically with the use of adoptive transplant, relapse of treatment-refractory disease remains a challenge. The experimental therapy is hypothesized to target transformed plasma B cells, based on preliminary work conducted at the Lankenau Institute. The therapy might also be useful to prevent the emergence of multiple myeloma from premalignant syndromes that can present clinically at earlier times, or to alleviate confounding effects of the cancer which include renal failure, anemia, infection, osteoporosis, polyneuropathy and other symptoms caused by tissue deposition of the antibodies produced by MM cells. This area is not strictly palliative, since it might

positively impact the quality of life of MM survivors benefiting from other therapies. The proof-of-concept work to be performed addresses gaps in knowledge about MM pathology and could rationalize clinical exploration of a new type of molecular targeted therapy for this deadly cancer.

Summary of Research Completed

Aim 1. Prepare and purify mAb suitable for in vivo testing. Biologics tested for cancer treatment must take care to prevent the introduction of inflammatory modifiers that may contaminate the preparation. *Mycobacterium avium paratuberculosis* (MAP) is the chief contaminant of concern in hybridoma cell cultures. Thus, RhoB mAb used in the preclinical trial was tested for MAP contamination using established methods. Using standard methods, we purified mAb from the 7F7 hybridoma clone (IgG2) originally characterized as immune inhibitory in the KBxN model of rheumatoid arthritis. We also purified mAb from a backup hybridoma clone, 9G5, which is slightly less specific than the 7F7 mAb for RhoB due to recognition of an overlapping but distinct epitope in C-terminal regions of RhoB, RhoA and Cdc42 which are very closely related overall in primary amino acid sequence (unpublished observations). In the mAb preparation prepared from the 7F7 hybridoma culture we obtained yields of ~100 mg that were characterized by protein concentration determinations, SDS-PAGE and ELISA assay, as compared to purified murine IgG obtained commercially (see Figures 1,2).

Hybridoma supernatent used to make the mAb preparations was subjected to PCR-based tests to screen for contamination of a panel of infectious agents, including mycoplasmas that are one common infection in hybridoma cultures. This test was contracted to a core facility, RADIL, which is located at the University of Missouri. The tests for each infectious agent were negative (see Figure 3). Both mAb preparations obtained were judged suitable for *in vivo* testing.

Aim 2. Test the effect of mAb treatment on the survival of MM cells in vitro. Based on accumulating information in our laboratory that RhoB mAb are not cytotoxic to neoplastically transformed murine B cells, nor normal human peripheral B cells, we elected not to conduct the proposed *in vitro* cell survival tests using multiple myeloma cells during the present grant period, but to proceed directly to *in vivo* testing. No progress is reported on this Aim.

Aim 3. Explore the safety and pharmacology of mAb in wild-type and MM-bearing mice. Genetic analysis of *rhoB* in the mouse indicates its loss does not affect normal physiology, but delivery of an inhibitory RhoB mAb may nucleate macromolecular complexes with pathologic effects. However, given the lack of evidence of toxicity of RhoB mAb as administered to unrelated mouse models being examined in parallel in our laboratory, that are not part of this project, including at doses and administration routes identical to that proposed in this project that exhibit therapeutic activity in the other models, we elected not to conduct the dose-ranging study to explore tox issues in this reporting period.

With regard to pharmacology, we benefitted from knowledge of therapeutic activity of 7F7 mAb in a model of autoimmune disease studied in an unrelated project in the laboratory, where it had been shown that genetic deletion of RhoB was sufficient to attenuate disease development

(unpublished observations). Specifically, we found that mAb 7F7 administered 0.5 mg/mouse as a bolus i.p. dose once every three weeks was sufficient to elicit efficacy in K/BxN mice, an established mouse model of rheumatoid arthritis. Based on this observation, for the present work we conducted an exploratory pharmacology experiment to examine the stability of mAb 7F7 in mouse serum at times after i.p. administration of wild-type BALB/c mice, the host for MOPC tumor engraftment, using a 'supermouse' design and an ELISA-based assay for mAb detection. The results confirmed that mAb 7F7 was detectable for up to 2 weeks after a single 0.5 mg i.p. injection. On the basis of this information, we elected to conduct the initial efficacy experiments in Aim 4 using a dose of 0.5 mg/mouse on a once-weekly schedule.

Aim 4. Evaluate the preclinical efficacy of mAb in MM-bearing mice. Efficacy studies employed a published model of multiple myeloma that uses MOPC315.BM plasmacytoma cells analogous to the human disease. This model system is a modification of the established MOPC315 cell system, adding a non-invasive fluorescence imaging reporter to a tumor subline selected for bone progression that further recapitulates progression of human multiple myeloma. In an initial test, we conducted a pilot experiment to verify progression in this tumor model in our hands, monitoring a described paralysis phenotype (due to bone marrow invasion). MOPC315.BM was engrafted to wild-type BALB/c mouse hosts by i.v. injection of $2x10^5$ cells into the tail vein (n=5) and progression was monitored by examining gait and overall survival. All mice in the pilot test exhibited signs of paralysis reflected initially in gait defects. Non-invasive imaging by bioluminescence confirmed tumor outgrowth associated with these defects (data not shown). Every subject in this pilot experiment succumbed to disease within 50 days of tumor engraftment (see Figure 3). This initial test confirmed the validity of the model system to compare the effects of RhoB mAb 7F7 on progression and survival to murine IgG antibody as a negative control.

In the first treatment trial, mAb 7F7 or purified mouse IgG was administered once weekly (0.5 mg/mouse) beginning the day after i.v. engraftment of $2x10^5$ MOPC315.BM cells into the tail vein of female BALB/c mice (n=10 per group). Survival was the experimental endpoint. We observed that tumor outgrowth was relatively slower compared to the tumor outgrowth in the non-treatment pilot test, with <50% of mice in either group succumbing to disease within 50 days of tumor engraftment (Figure 4). It was unclear whether this difference reflected the addition of weekly antibody treatment or the effects of repeated i.p. injection (the only variables altered in the design from the pilot test), or another unaccounted variable in this trial. In any case, in terms of outcome we observed no significant difference in survival between the experimental and control groups (Figure 4). In ongoing and future work, we will explore dose range and combination regimens, as proposed in alternate approaches in the project, along with a related single-agent trial in a solid tumor model of breast cancer where genetic proof-of-concept exists, based on recent work from our group in collaboration with L Benjamin at Lilly/ImClone.

Table 1. PCR analysis of hybridoma supernatants for cultured infections. Data provided by a core facility at the University of Missouri (RADIL) is shown. Each evaluation included a positive control (not shown). Supernatents were mixed for study. 6G/8I3 is an unrelated sample.

PCR EVALUATION:

Specimen: cells	7F7-2/9G5-4	6F/8I3
Ectromelia	-	-
EDIM	-	-
Hantaan	-	-
K virus	-	-
LCMV	-	-
LDEV	-	-
MAD	-	-
mCMV	-	-
MHV	-	-
MNV	-	-
MPV	-	-
MTV	-	-
MVM	-	-
Mycoplasma pulmonis	-	-
Mycoplasma sp.	-	-
Polyoma	-	-
PVM	-	-
REO3	-	-
Sendai	-	-
TMEV GDVII	-	-

(LEGEND: + = positive - = negative id:id = pooled sample blank = test not performed equ = equivocal NT or blank = no test performed sus = suspect wps = weak positive)

Figure 1. Evaluation of purified RhoB mAb by SDS-PAGE. Coomassie-stained gel shows IgG heavy and light chains obtained after purification from hybridoma supernatant by standard methods. Lane 1, gel marker; Lanes 2 and 5, mAb 7F7; Lanes 3 and 6, mAb 9G5; Lanes 4 and 7, control mouse IgG (commercially obtained).

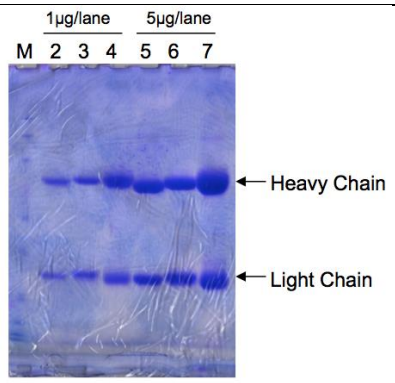


Figure 2. Evaluation of purified lead mAb 7F7 by ELISA assay. Serial dilutions of purified IgG were captured by sheep anti-mouse IgG. Purified antibody was detected with HRP-conjugated goat and mouse IgG and ABTS substrate. 7F7 was detected in a similar fashion as control mouse serum IgG1 (control ms), suggesting the 7F7 preparation was predominantly IgG.

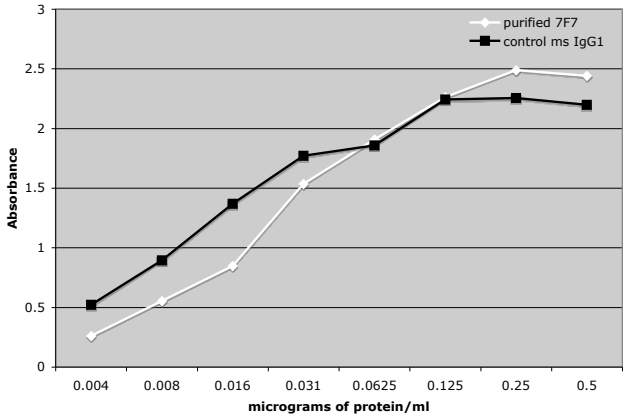


Figure 3. Confirmation of the model system. Tumors were initiated in 8 week old BALB/c female mice (n=5; Jackson Laboratories) by i.v. injection of 2x10e5 MOPC315 BM-Luc cells in 100 µl PBS into the tail vein. Mice were monitored as described in the text.

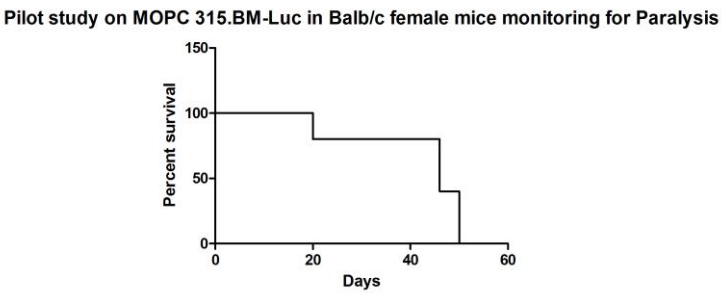
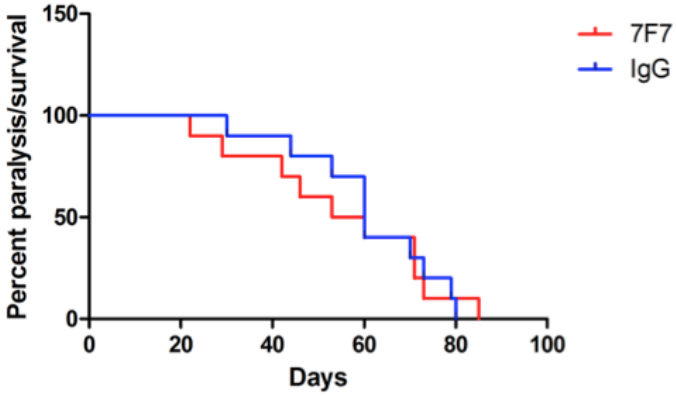


Figure 4. Pilot trial of RhoB mAb in MOPC315.BM-bearing mice. Tumors were initiated as before (n=5 per group). The following day, RhoB mAb 7F7 or control mouse Ig was administered i.p. at 0.5 mg/mouse with similar dosing once each week until the end of the experiment. Mice were monitored non-invasively once within a month of tumor cell injection by bioluminescence imaging to confirm tumor engraftment (not shown).



Comparison of Survival Curves	
Log-rank (Mantel-Cox) Test	
Chi square	0.04004
df	1
P value	0.8414
P value summary	ns
Are the survival curves sig different?	No
Gehan-Breslow-Wilcoxon Test	
Chi square	0.4132
df	1
P value	0.5204
P value summary	ns
Are the survival curves sig different?	No
Median survival	
7F7	56.50
IgG	60.00
Ratio	0.9417
95% CI of ratio	0.5254 to 1.358
Hazard Ratio	
Ratio	1.103
95% CI of ratio	0.4211 to 2.891