

AWARD NUMBER: W81XWH-15-1-0141

TITLE: Enhancing Immune Checkpoint Inhibitor Therapy in Kidney Cancer

PRINCIPAL INVESTIGATOR: Hans-Joerg Hammers

CONTRACTING ORGANIZATION: UT Southwestern Medical Center, Dallas, TX

REPORT DATE: January 2021

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE January 2021		2. REPORT TYPE Final		3. DATES COVERED 30Sep2015-30Sep2020	
4. TITLE AND SUBTITLE Enhancing Immune Checkpoint Inhibitor therapy in Kidney Cancer				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-15-1-0141	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Hans Hammers, M.D., Ph.D. E-Mail: hans.hammers@utsouthwestern.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) UT Southwestern Medical Center 5323 Harry Hines Blvd. Dallas, TX 75390-7208				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The purpose of this work is to develop strategies to enhance immune checkpoint inhibition in kidney cancer. The work is designed to test different strategies to induce or enhance the abscopal in a kidney cancer model by combining ablative techniques or TLR agonists with PD1 inhibitors. Animal Protocol is now ACURO and UTSW approved. Work will start as soon as no cost extension has been approved.					
15. SUBJECT TERMS Kidney cancer, Immunotherapy, abscopal effect					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 9	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	2
2. Keywords.....	2
3. Accomplishments.....	2
4. Impact.....	7
5. Changes/Problems.....	7
6. Products.....	7
7. Participants & Other Collaborating Organizations.....	7
8. Special Reporting Requirements.....	7

1. INTRODUCTION:

The purpose of this research is to test therapeutic strategies to make immune checkpoint inhibition more effective in an animal model of kidney cancer. Our plan was to combine focally ablative techniques such as high dose radiation or cryotherapy with PD1 immune checkpoint inhibition and assess the abscopal effect, i.e. the growth of tumors at a distant, untreated site. Additionally, we tested the effect of TLR agonist administration in this model and its combination with focally ablative techniques.

2. **KEYWORDS:** kidney cancer, immunotherapy, PD1, TLR, abscopal, radiation,

3. ACCOMPLISHMENTS:

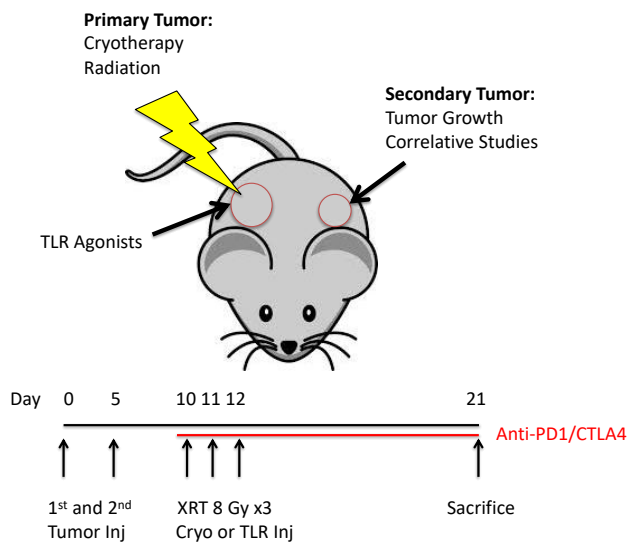
Funding was transferred in mid/fall 2017.

Over the course of the grant we have accomplished the following goals:

Impact of radiation and PD1 Immunotherapy in the RENCA murine renal cell carcinoma model:

Aim 1: To compare the efficacy of tumor ablative strategy, High-dose radiation regarding their ability to induce an effective tumor response when combined with immune checkpoint inhibitor blockade.

Hypothesis: Destruction of tumor cells with high-dose radiation will release tumor antigens and an inflammatory response which can synergize with PD1 inhibition in a murine renal cell carcinoma model.

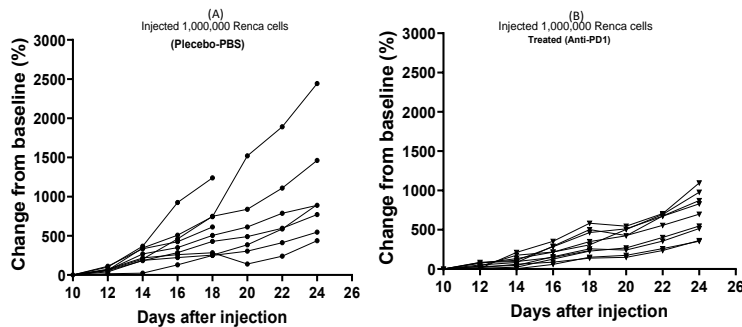


The primary goal was to study the treatment effect on the directly treated primary tumor and the potential abscopal effect in the secondary tumor on the opposite side of the mouse.

RESULTS:

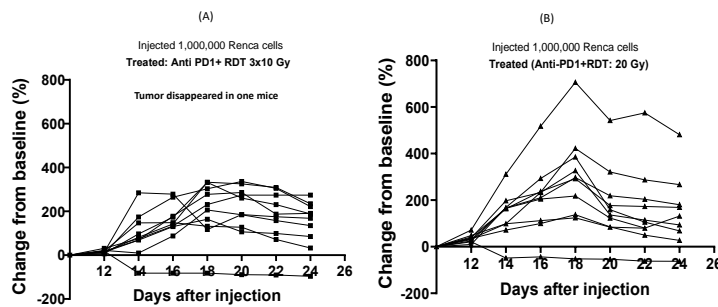
PD1 Monotherapy has little effect on primary tumor growth, i.e. minimal growth delay, no tumor disappearances, supporting the choice of this model as PD1 refractory model suitable for development of better combination therapies.

Tumor vs Anti PD1 treatment (Primary Tumor)



Radiation in combination with PD1 inhibition increases the anti-tumor effect on primary tumor. Fractionated radiation (3x 10 Gy) was found to be slightly more effective than 1 dose of 20 Gy. Studies with cryoablation yielded no noticeable benefit in the abscopal tumor and therefore our studies focused on the combination with radiation therapy.

Anti PD1+RDT 3X10 Gy vs Anti PD1+RDT 20 Gy (Primary Tumor)



Importantly, an apparent response and improved tumor control was observed in the secondary tumor consistent with abscopal effects in the non-irradiated tumors. The tumors in 2 mice treated with fractionated radiation disappeared.

Anti PD1+RDT 3X10 Gy vs Anti PD1+RDT 20 Gy (Secondary Tumor)

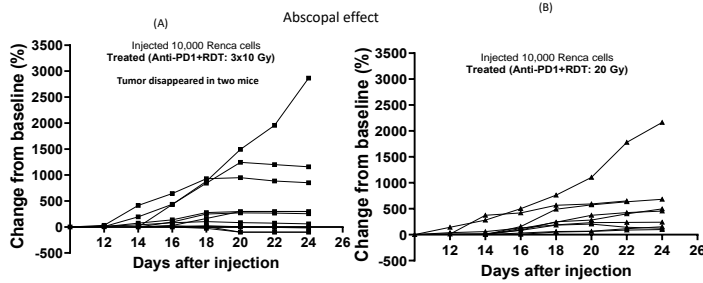


Figure showing the combination effects radiation treatment plus Anti PD1 (A) 10 Gy radiation on 12th, 13th and 14th Days after RENCA cell injection (3 times) (B) 20 Gy Radiation on 12th day (one time)

Aim 2: To test the hypothesis that local administration of clinically relevant toll-like receptor (TLR) agonists will synergize with PD-1 blockade in mediating local and systemic anti-tumor immune responses.

Hypothesis: These experiments will use the same orthotopic RCC models as in Aim 1, but instead TLR agonists such as CpG (TLR-9), Imiquimod (TLR-7) and MPLA (TLR-4) will be administered intra-tumorally in mice treated concomitantly with PD-1 blocking antibodies.

RESULTS:

Direct intratumoral injections have minimal effects with Imiquimod (TLR7), but are clearly more pronounced with CpG ODN (TLR9) and MPLA (TLR4).

Primary Tumors: Imiquimod vs CpG ODN vs MPLA

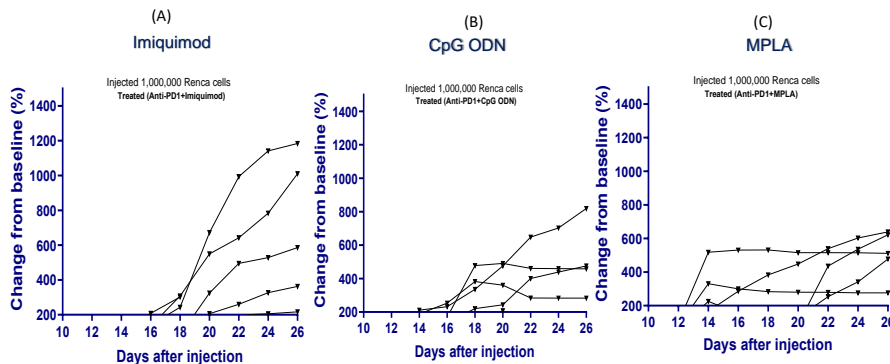
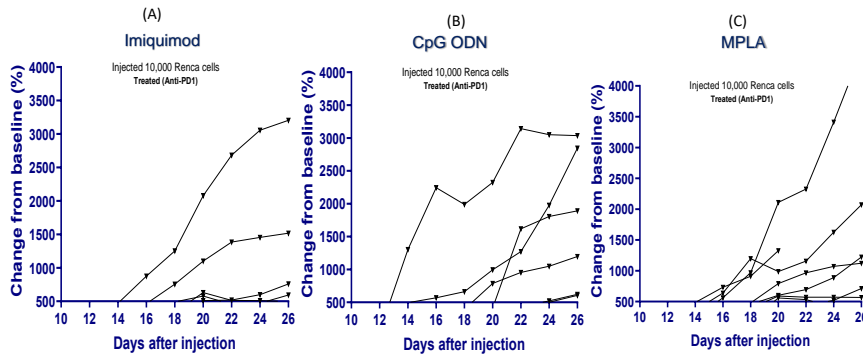


Figure showing the ability of three TLR agonists (IT) 12th, 14th and 16th Days after RENCA cell injection plus Anti PD1 (3X/week) (A) Imiquimod (TLR7)(B) CpG ODN (TLR 9) (C) MPLA (TLR 4)

Surprisingly, the abscopal effect of TLR agonism with PD1 blockade appears inferior to radiation/PD1 inhibition.

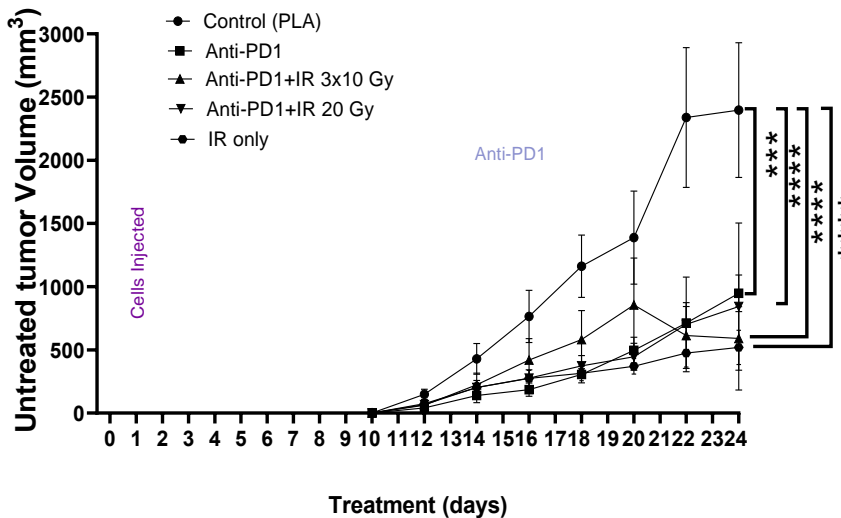
Secondary Tumors: Imiquimod vs CpG ODN vs MPLA



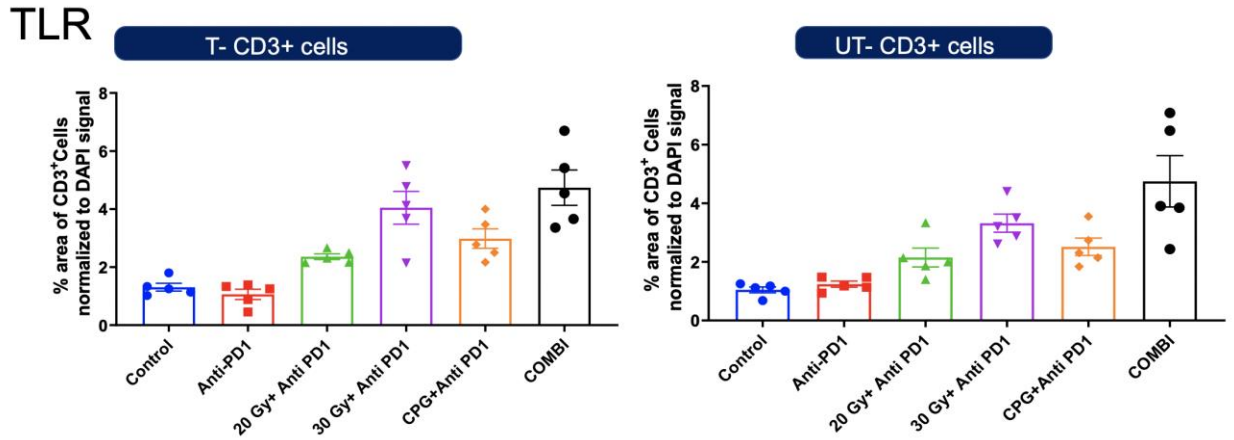
Triple Combination Therapy:

For the final animal studies we've combined radiation treatment with PD1 inhibition and CpG injection of the irradiated tumor.

Combination: Irradiated tumor



While the effect of triple therapy on the primary tumor were modest in comparison the PD1/XRT, the triple combination with the CpG agonist had a significant effect on the abscopal tumors suggesting a significantly enhanced systemic treatment effect.



Immunohistochemistry studies assessing for CD3 positive cells demonstrate significant increases in tumor infiltrating lymphocytes for the combination in irradiated but also distant tumors supporting the mechanistic underpinnings of an abscopal effect.

SUMMARY:

- We evaluated antitumor effects on RENCA tumor-bearing mice.
- A significant decrease in the tumor burden was observed in the radiation treated but not in the anti-PD-L1 alone treatment group compared with the control group.
- The present results indicated that tumors grew significantly less in mice treated with radiation and some mice showed abscopal effects in radiated mice.
- Tumor disappeared in 3 mice receiving fractionated radiation (3x10Gy) and was chosen as the most effective radiation regimen for future/ongoing studies
- TLR agonist treatment was least effective in Imiquimoid (TLR 7) treated mice and most effective in CpG ODN (TLR9) .
- Tumor infiltrating lymphocytes trended to follow the antitumor effects in our animal model

4. **IMPACT:**

The data from this preclinical model supplements emerging data from our RADVAX clinical (NCT03065179) which combined high dose stereotactic radiation with immune checkpoint inhibition. Data from the trial has been presented at GU ASCO 2020.

Data from the pre-clinical studies are currently informing the design of follow-up studies.

5. **CHANGES/PROBLEMS:**

Our studies proceeded well. We are finishing correlative studies and are preparing a manuscript.

6. **PRODUCTS:**

Nothing to report

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:**

None

8. **SPECIAL REPORTING REQUIREMENTS:**

None