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TITLE: Enhancing Immune Checkpoint Inhibitor Therapy in Kidney Cancer

PRINCIPAL INVESTIGATOR: Hans-Joerg Hammers

CONTRACTING ORGANIZATION: UT Southwestern Medical Center

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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.					
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Table of Contents

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

1. **INTRODUCTION:** Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

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 is to combine focally ablative techniques such as 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 will test the effect of TLR agonist administration in this model and look at combination with focally ablative techniques.

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

3. **ACCOMPLISHMENTS:**

Funding was transferred in mid/fall 2017.

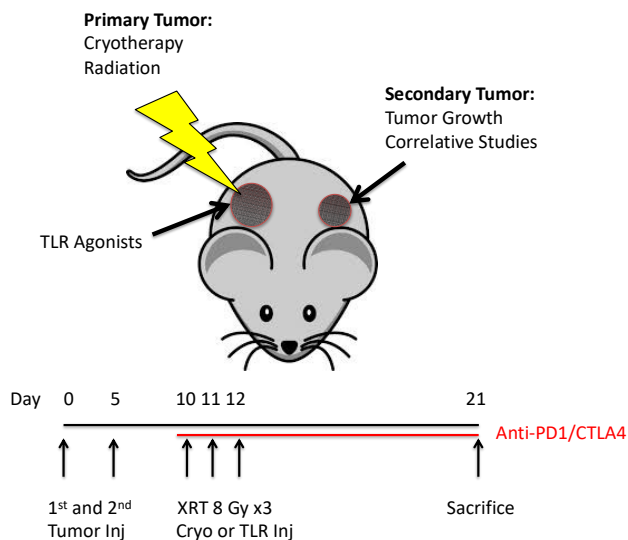
We have obtained UTSW and ACURO animal protocol approval.

In 2018/2019 we have accomplished the following goals:

1. 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:

Tumor vs Anti PD1 treatment (Primary Tumor)

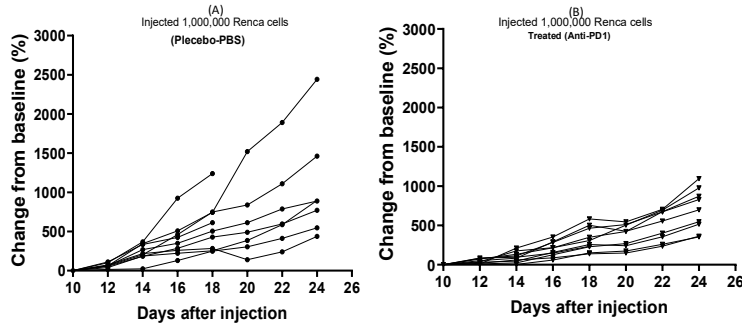


Figure showing the effects (A) Placebo-PBS (B) Anti-PD1 3X/ Week (10th, 13th, 14th days after RENCA cell injection)

PD1 Monotherapy has little effect on primary tumor growth, i.e. minimal growth delay, no tumor disappearances

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

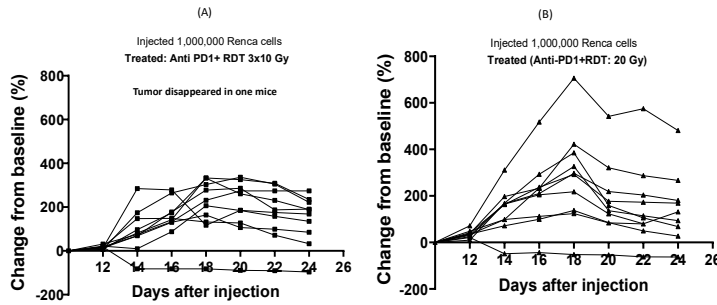


Figure showing the combination effects radiation treatment plus Anti PD1 (A) 10 GY radiation on 12th, 13th and 14th Days after RENCA cell injection (3 times) (B) 20 GY Radiation on 12th day (one time)

Effect of radiation plus PD1 inhibition increases effect primary tumor. Fractionated radiation (3x 10 Gy) was found to be slightly more effective than 1 dose of 20 Gy.

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

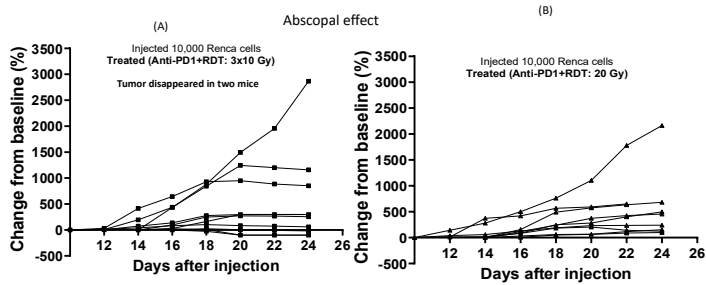


Figure showing the combination effects radiation treatment plus Anti PD1 (A) 10 GY radiation on 12th, 13th and 14th Days after RENCA cell injection (3 times) (B) 20 GY Radiation on 12th day (one time)

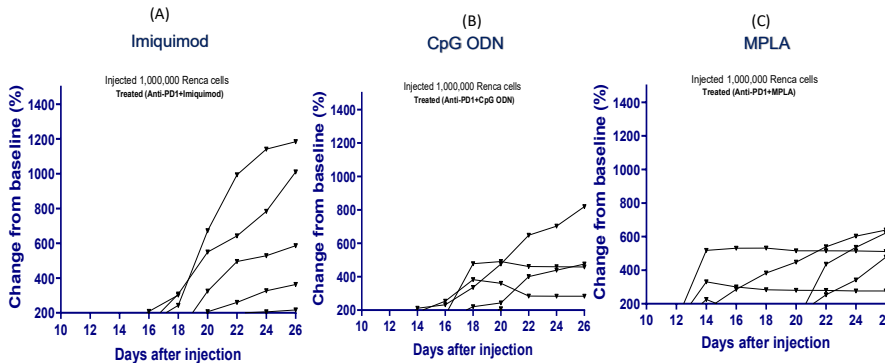
This finding is reproduced in the secondary tumor with apparent abscopal effects in the non-irradiated tumors. The tumors in 2 mice treated with fractionated radiation disappeared.

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 intratumorally in mice treated concomitantly with PD-1 blocking antibodies.

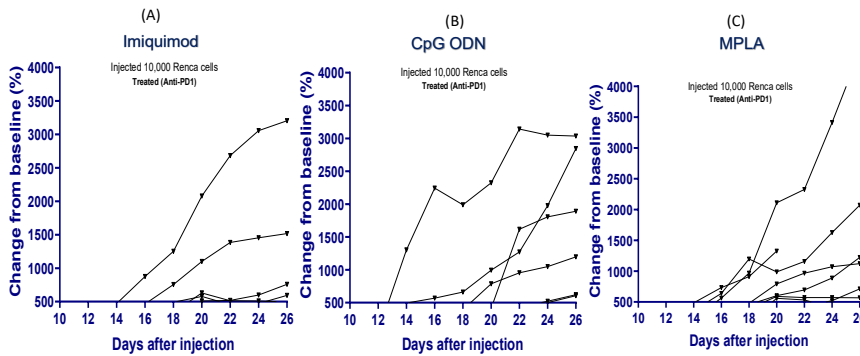
RESULTS:

Primary Tumors: Imiquimod vs CpG ODN vs MPLA



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

Secondary Tumors: Imiquimod vs CpG ODN vs MPLA



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

Plans: We are currently combining TLR agonist with radiation and PD1 inhibition in ongoing animal studies.

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 was 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) .
- Additional end points will changes in tumor infiltrating lymphocytes by Immunohistochemistry/flow cytometry.

4. IMPACT:

The data from this preclinical model supplements emerging data from our RADVAX clinical (NCT03065179) which combines high dose stereotactic radiation with immune checkpoint inhibition. Data from the trial will be 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 are proceeding well. We are currently performing various correlative work with tumor immune-fluorescence staining and flow cytometry of the immune infiltrate.

6. PRODUCTS:

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS:

None

8. SPECIAL REPORTING REQUIREMENTS:

None