AWARD NUMBER: W81XWH-21-1-0433

TITLE: Low-Dose Radiation Ex Vivo Reprogrammed/Activated CAR T Cells Targeting B7-H3 on Prostate Cancer

PRINCIPAL INVESTIGATOR: Xinhui Wang

CONTRACTING ORGANIZATION: Massachusetts General Hospital

REPORT DATE: OCTOBER 2022

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
OCTOBER 2022	ANNUAL	1SEPT2021 - 31AUG2022

4. TITLE AND SUBTITLE

Low-Dose Radiation Ex Vivo Reprogrammed/Activated CAR T Cells Targeting B7-H3 on Prostate Cancer

5a. CONTRACT NUMBER
W81XWH-21-1-0433

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

Xinhui Wang

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Massachusetts General Hospital

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:**

Metastatic prostate cancer (mPCa), which can be subdivided into hormone-sensitive (mHSPC) and castration-resistant PCa (mCRPC), is the lethal form of PCa , with a 5-year survival rate of 30%. Current therapies can prolong survival for mPCa patients; however, resistance invariably develops and eventually causes death. To address this unmet clinical need, we have recently developed a novel chimeric antigen receptor (CAR) T cell immunotherapy (CAR T therapy) by reprogramming/activation of CAR T cells that recognize B7- H3(CD276), an immune checkpoint which is almost uniformly expressed on differentiated (bulk) PCa cells and PCa stem cells (PCSPs), which can cause therapeutic resistance. B7-H3 expression increases in higher Gleason score prostate cancer and with progression to metastatic and castration-resistant disease, and is correlated with cancer-specific mortality. Conversely, B7-H3 expression on normal tissue is minimal. Low-dose radiation (IR) by upregulating NF-κB -stemness gene pathway empowers CAR T cells (IR CAR T) capable of producing a robust and long lasting anti-tumor activity. The IR B7-H3 CAR T compared to non-IR CAR T shows much increased potency in 1) in vitro killing of differentiated PCa and PCSCs in human PCa cell lines, and 2) in vivo inhibiting PCa or breast cancer xenograft growth, as measured by complete or substantial tumor regression and long-term survival in the absence of toxicity.

15. SUBJECT TERMS: NONE LISTED

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	**24**	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

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

The goal of this proposal is to test the hypothesis that Low-dose irradiation (IR) *ex vivo* reprogrammed/activated B7-H3 CAR T cells significantly prolong survival of mice bearing metastatic prostate cancer (mPCa), including metastatic hormone-sensitive prostate cancer (mHSPC) and metastatic castration-resistant prostate cancer (mCRPC), by eradicating differentiated bulk prostate cancer (PCa) cells and prostate cancer stem cells (PCSCs).

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Radiation, reprogrammed/activated CAR T cells, B7-H3, metastatic prostate cancer

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

1: Optimize the strategy of using low-dose IR to reprogram/activate B7-H3 CAR T cells and phenotypically and functionally characterize the reprogrammed CAR T cells.

2: Determine efficacy and safety of low-dose IR *ex vivo* reprogrammed/activated B7-H3 CAR T cells derived from mPCa patients to prolong survival of mice bearing human mHSPC or mCRPC cell-derived orthotopic xenografts.

3: Assess expression frequency and intensity of the B7-H3 epitope recognized by the B7-H3-specific mAb 376.96 used to make the B7-H3 CAR T cells, on PCa cells and PCSCs present in tissue samples from mPCa patients.

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

Specific aim 1: Optimize the strategy of using low-dose IR to reprogram/activate B7-H3 CAR T cells and phenotypically and functionally characterize the reprogrammed CAR T cells.

Specific aim synopsis

In this aim, we titrated the doses and post-duration of irradiation (IR) on reprogramming/activating B7-H3 CAR T cells; and characterized phenotypically and functionally the reprogrammed CAR T cells under the identified optimized IR dose and duration post-IR.

***Subtask 1*:** Optimize strategy for *ex vivo* IR-reprogramming of B7-H3 CAR T cells

IR dose titration on proliferation and killing capacity of CAR T cells

We did many times of IR dose titrations ranging from 0-1 Gy to identify the optimal IR dose for enhanced killing activity while with minimum reduced cell proliferation of CAR T cells. We found that i) irradiated CAR T cells with a single dose of 0.5Gy exhibit most effective killing efficacy on both PCa cell lines PC3 and LNCap than any other tested IR doses (**Fig1**), the enhanced cytotoxicity of target cells by the dose of IR at 0.5 Gy of CAR T cells was confirmed by using another CRPC cell line DU145 (**Fig2**) ii) the increased killing efficacy by irradiated CAR T cells (0.5Gy) of the target cells PC3 is time dependent: post-3 days of IR showed to be optimal compared to other tested days(**Fig3).** Furthermore, we found that i) CAR T cell proliferation was reduced by about 20% after a single dose of IR at 0.5Gy compared to 0 Gy (**Fig4**), ii) CAR T cell proliferation was gradually increased from days 2-4 regardless of being irradiated, although irradiated (0.5Gy) CAR T cells had lower proliferation than non-irradiated ones (**Fig5**).

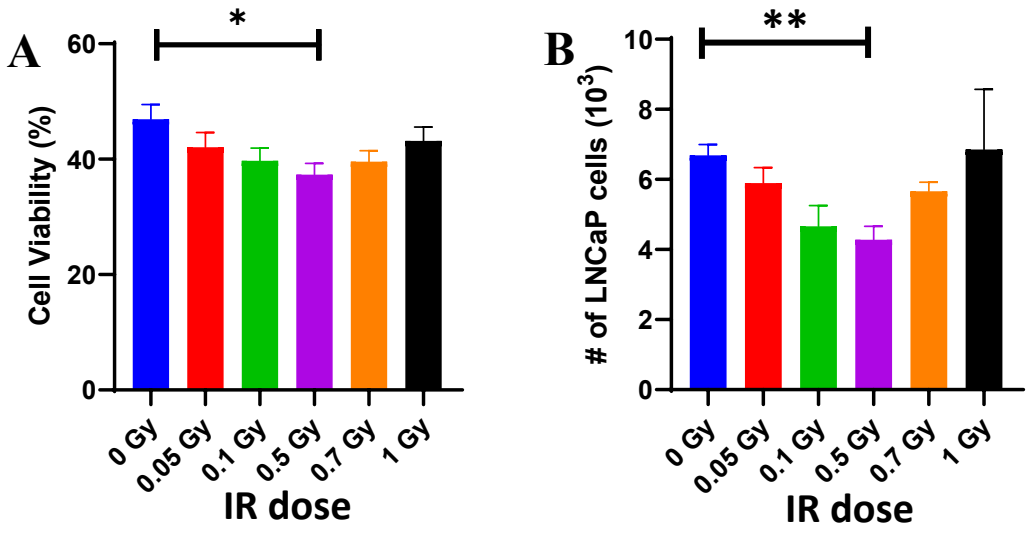

Fig1. A single dose of 0.5Gy to reprogram B7-H3 CAR T cells is optimal in enhancing killing B7-H3$^+$ target PCa cells *in vitro*. To reprogram CAR T cells, B7-H3 CAR cells were irradiated at indicated doses. Following a 3-day culture and expansion, the reprogrammed CAR T cells were co-cultured with PC3 cells or LNCaP PCa cells at the effector: target (E:T ratio) 1:2 for 48 hrs. CAR T cells in the cell suspension were removed, and the viability of adherent target cells was quantitated by MTT assays for PC3 cells **(A)** or viable target cells were stained with B7-H3-specific antibody and quantified by counting beads via flow analysis **(B).** * p<0.05, ** p<0.01.

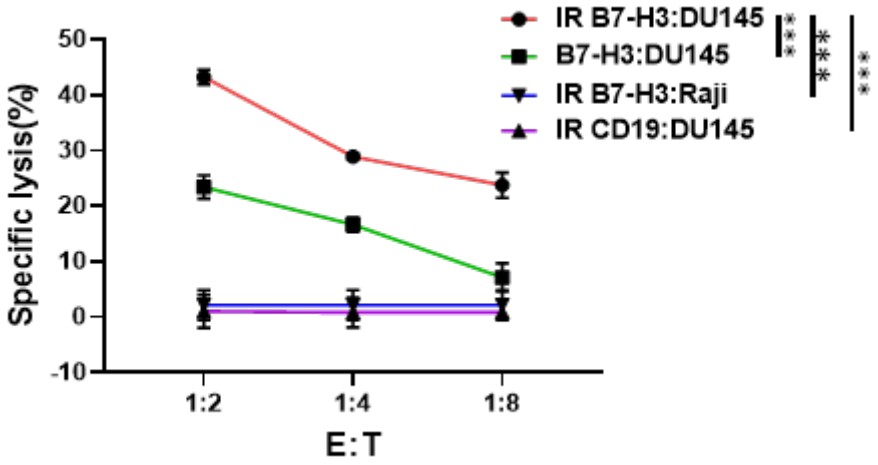

Fig2. IR reprogrammed B7-H3 CAR T cells are much more potent than B7-H3 CAR T cells in killing B7-H3$^+$ target PCa cells *in vitro*. IR (0.5Gy) reprogrammed CAR T cells (IR B7-H3 CAR T) or non-irradiated B7-H3 CAR T (**B7-H3 CAR T**) cells were co-cultured with DU145 cells at indicated E:T ratios for 24hrs. CAR T cells in the cell suspension were removed, and the

viability of adherent target cells was quantitated by MTT assays. IR CD19 CAR T cells and B7-H3-Raji cells were used as a specificity control. The viability of cell populations treated with IR B7-H3 CAR T vs B7-H3 CAR T vs IR CD19 CAR T is shown. *** p<0.001

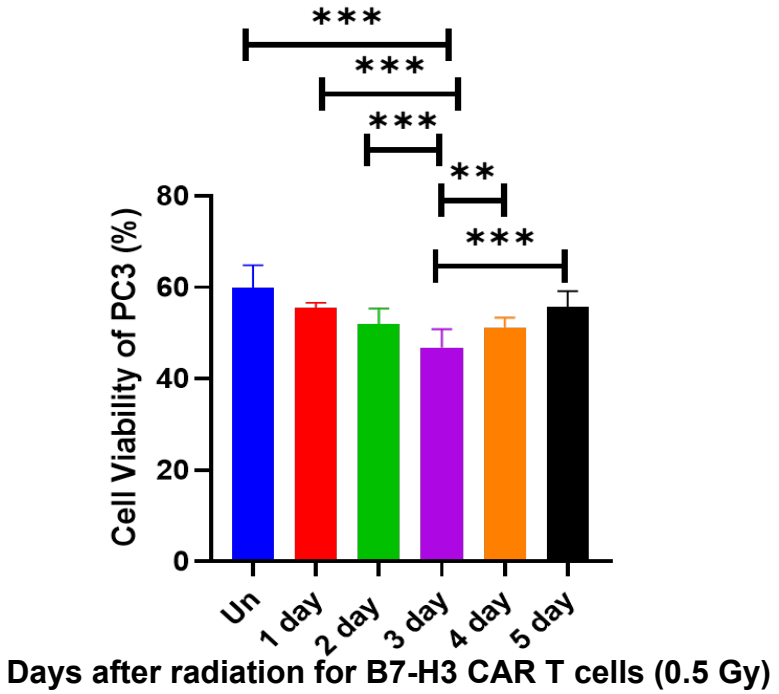

Fig3. Three days of culture and expansion post-IR is optimal in enhancing killing capacity of B7-H3$^+$ target PCa cells *in vitro*. IR (0.5Gy) irradiated CAR T cells were cultured and expanded at the indicated times, then the reprogrammed CAR T cells were co-cultured with PC3 cells at the E:T ratio 1:2 for 48 hrs. The viability of adherent target cells was quantitated by MTT assays for PC3 cells. ** p<0.01, *** p<0.001

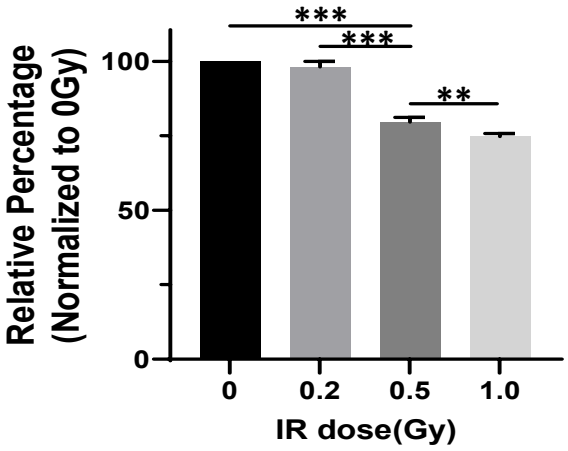

Fig4. Low–dose IR decreased CAR T cell proliferation in vitro. To reprogram CAR T cells, B7-

H3 CAR cells were irradiated at indicated doses. Following a 3-day culture and expansion, the number of viable CAR T cells was counted using the Trypan blue exclusion method. **p<0.01. *** p<0.001

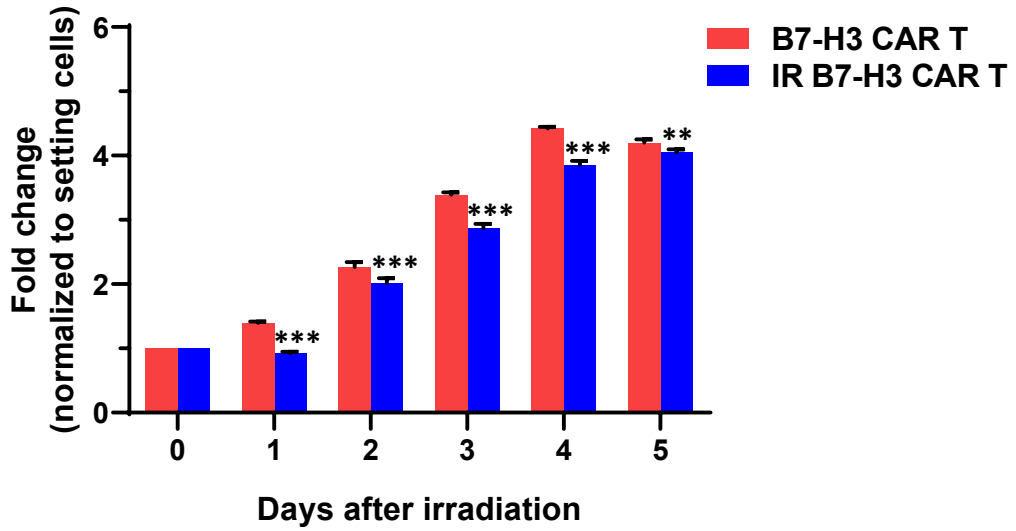

Fig5. CAR T cell proliferation post-low dose IR is time dependent. IR (0.5Gy) irradiated CAR T cells were cultured and expanded at the indicated times. Then the reprogrammed CAR T cells were counted using the Trypan blue exclusion method **p<0.01. *** p<0.001

***Subtask 2*:** Phenotypically characterize *ex vivo* IR -reprogramed B7-H3 CAR T cells for detection of early stem T cells.

We performed experiments to detect early stem T cells after ex vivo IR of CAR T cells with different stem cell markers such as CD62L, CD45RA, CD45RO, CCR7 by flow analysis. However, no difference in early stem T cells defined by these markers was found between irradiated vs non-irradiated CAR T cell populations. Thus, we changed the methodology to real-time cycles of PCR to detect a broad array of Human Stem Cell Transcription Factors using RT² Profiler PCR Array (Qiagen Cat # PAHS-501ZF-2) in irradiated vs. non-irradiated CAR T cells. As shown in Fig6, 22/84 Stem Cell Transcription Factors were increased in irradiated CAR T cells compared to non-irradiated CAR T cells (**Fig6**).

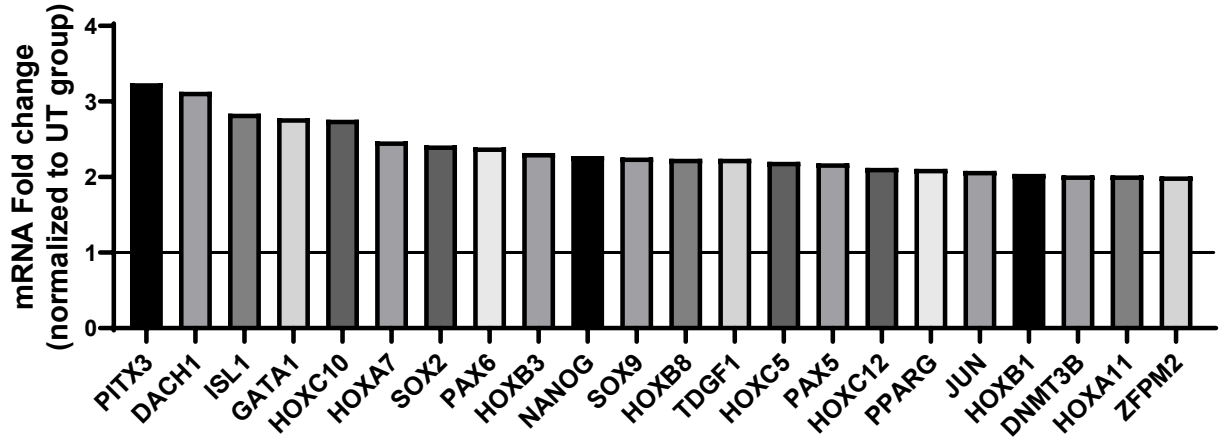

Fig6. IR reprogrammed CAR T cells had increased human stem cell transcription factors. To detect expression levels of human stem cell transcription factors, total RNA was extracted from IR reprogrammed (0.5 Gy, followed 3 days of culture and expansion) or non- IR reprogrammed CAR T cells for quantitative real-time PCR using RT² Profiler PCR Arrays. Based on mRNA fold changes, 22/84 stem cell transcription factors were increased in IR reprogrammed CAR T cells compared to non-reprogrammed CAR T cells.

Subtask 3: Functionally characterize *ex vivo* IR -reprogramed B7-H3 CAR T cells for pro-immune cytokine production.

We quantified *ex vivo* IR-reprogramed B7-H3 CAR T cells for pro-immune cytokine production in irradiated CAR T cell lysate and supernatant (SNT) collected after coculture with the target cancer cells using a customized human 25-plex Luminex panel (Thermo Fisher Scientific) containing the following analytes: CCL11, GM-CSF, Granzyme B, HSP60, IFNα, IFNγ, IL-1, IL-3, IL-4, IL-6, IL7, IL8 (CXCL8), IL-9, IL-10, IL-12p70, IL-15, IL-17A, IL-18, CXCL10, M-CSF, CXCL9, CCL3, CCL4, CCL5, TNFα. Data were acquired on a MAGPIX instrument and analyzed using the ProcartaPlex analysis app. We found that i) increased only IL-15 was increased in IR-reprogramed B7-H3 CAR T cell lysate compared to non-reprogramed B7-H3 CAR T cell lysate and increased released IFNγ, IL-7, IL-15 and CXCL10 and reduced released immunosuppressive IL-10 in the SNT of IR-reprogramed B7-H3 CAR T and target cell -coculture compared to that of non-reprogramed B7-H3 CAR T and target cell -coculture (**Fig7**). In addition, we evaluated cytotoxic T cell function using the LEGENDplex™ Human CD8/NK Panel (Biolegend) which allows

simultaneous quantification of released soluble IL-2, IL-4, IL-10, IL-6, IL-17A, TNF-α, sFas, sFasL, IFN-γ, granzyme A, granzyme B, perforin and granulysin - a panel of 13 human protein-effector profile of T cells. The results showed that *ex vivo* IR-reprogramed B7-H3 CAR T cells had a superior cytotoxic cell profile than non- IR-reprogramed CAR T cells (**Fig8**).

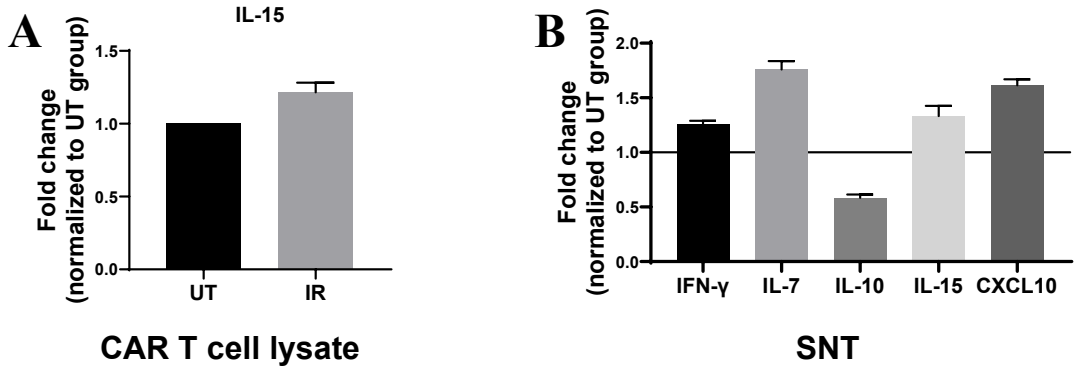

Fig7. IR reprogrammed CAR T cells cocultured with B7-H3+ target cells produced higher pro-inflammatory cytokines/chemokine. IR reprogrammed B7-H3 CAR T cells were co-cultured with B7-H3+ HCT116 cells (E:T=1:1) for 48 hours, followed by collecting CAR T cells (when the targets were all killed) and cell culture supernatant (SNT). Then the CAR T cell lysate (**A**) and SNT (**B**) obtained from IR reprogrammed vs. non- IR reprogrammed B7-H3 CAR T cells were measured for cytokines and chemokines using a customized human 25-plex Luminex panel detecting 25 cytokines and chemokines. $^{**}p<0.01$. $^{***}p<0.001$

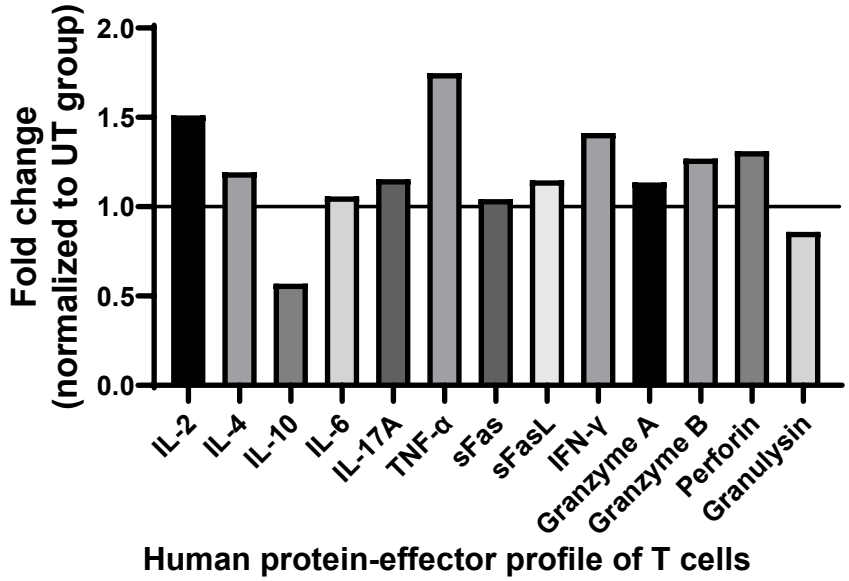

Fig8. IR reprogrammed CAR T cells possessed a superior effector cell profile. IR reprogrammed B7-H3 CAR T cells were cocultured with B7-H3+ HCT116 cells for 48 hours,

followed by collecting cell culture supernatant (SNT). Then the SNT obtained from coculture using IR reprogrammed vs. non- IR reprogrammed B7-H3 CAR T cells were analyzed for released soluble 13 human protein-effector profile of T cells. Compared to non- IR reprogrammed B7-H3 CAR T cells (as untreated (UT), IR reprogrammed B7-H3 CAR T cells showed a stronger effector cell profile.

Aim2: Determine efficacy and safety of low-dose IR *ex vivo* reprogrammed/activated B7-H3 CAR T cells derived from mPCa patients to prolong survival of mice bearing human mHSPC or mCRPC cell-derived orthotopic xenografts.

Specific aim synopsis

In this aim, we have successfully made and IR reprogrammed CAR T cells derived from normal donors and a patient's PBMCs. Using these CAR T cells, first, we found IR reprogrammed CAR T cells, either derived from a normal donor or a patient with CRPC, were more effective in treating orthotopic PCa xenografts in mice. Second, we detected increased infiltrated CAR T cells in tumors treated with IR reprogrammed B7-H3 CAR T cells than that treated with non-IR reprogrammed B7-H3 CAR T cells.

***Subtask 1*:** Making patient-derived and normal donor-derived B7-H3 CAR T cells and CD19 CAR T as controls

We successfully developed patient-derived and normal donor-derived B7-H3 CAR T cells and CD19 CAR T as controls. These CAR T cells were used for in vivo experiments shown below.

***Subtask 2*:** Establish orthotopic highly spontaneously metastatic HSPC and metastatic CRPC mouse xenograft models

We have established an orthotopic highly spontaneously metastatic CRPC mouse xenograft model with PC3 cells (**Fig9**).

***Subtask 3*:** Testing efficacy of IR reprogrammed/activated B7-H3 CAR T cells derived from mPCa patients effectively control growth and metastasis of orthotopic human PCa cell line-derived xenografts

We have collected blood from 3 patients with metastatic CRPC and isolated PBMCs. We made CAR T cells from one of the patients' PBMCs and stored them in an LN2 freezer. In a pilot experiment, we found that low-dose irradiation ex vivo reprogrammed patient-derived B7-H3 CAR T cells are much more potent than B7-H3 CAR T cells in controlling orthotopic human PC3 cell line-derived xenograft growth in NSG mice (**Fig9**).

***Subtask 4*:** Examining the impact of IR reprogramming/activation on PCSCs, B7-H3 CAR T cell infiltration and activation in mPCa cell-derived xenografts obtained from B7-H3 CAR T cell-treated mice

In order to collect enough tumor tissues, we delayed CAR T treatment in mice bearing PC3 cell line-derived orthotopic PCa xenografts in NSG mice, and one week later, we euthanized the mice to collect tumors for analysis. We found that IR reprogrammed B7-H3 CAR T cells (derived from a normal donor) are more effective in controlling tumor growth than non- IR reprogrammed B7-H3 CAR T cells (**Fig10**). Importantly, significantly increased tumor infiltrated CAR T cells were detected in tumors treated by IR reprogrammed B7-H3 CAR T cells than by non-IR reprogrammed B7-H3 CAR T cells (**Fig11**).

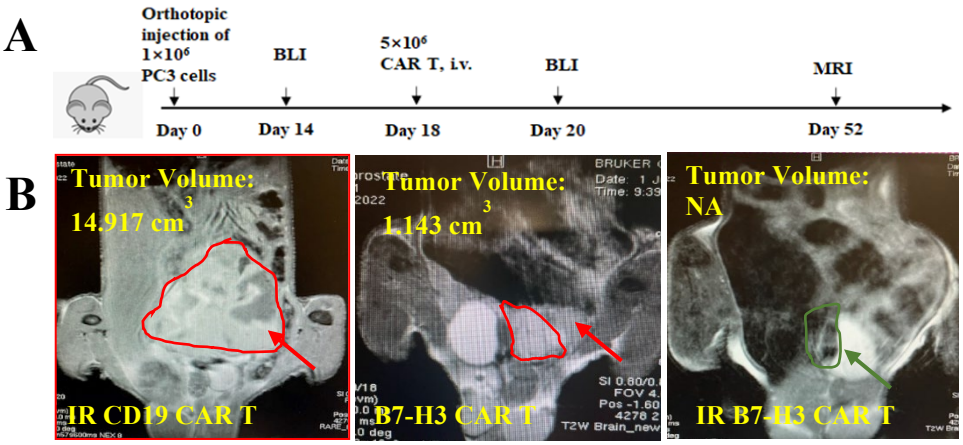

Fig9. Low-dose irradiation ex vivo reprogrammed B7-H3 CAR T cells were much more

potent than B7-H3 CAR T cells in controlling orthotopic human CRPC PCa xenograft growth. On Day 0, luciferase labeled CRPC PC3 cells were orthotopically (through surgery) inoculated into peripheral zone of prostate of 8-wk-old male NSG mice. After tumor detection by BLI, the mice were divided into 3 groups of 2 mice each and the treatment was initiated (A). The B7-H3 CAR T cells were made with PBMC from a patient with mCRPC. The patient-derived CAR T cells were either *ex vivo* reprogrammed by irradiating them with IR (0.5Gy X-ray) (namely, IR CAR T) or non-IR treated (CAR T). Representative tumor burden detected by MRI is shown: B7-H3 CAR T inhibited PCa growth vs. IR CD19 CAR T (PC3 cells are B7-H+CD19-), while complete tumor regression was detected in IR B7-H3 treated mice. **Red cycle**: tumor area. **Green cycle**: prostate. Four-dimensional(D) MRI measured tumor volumes are shown on top-left (**B**).

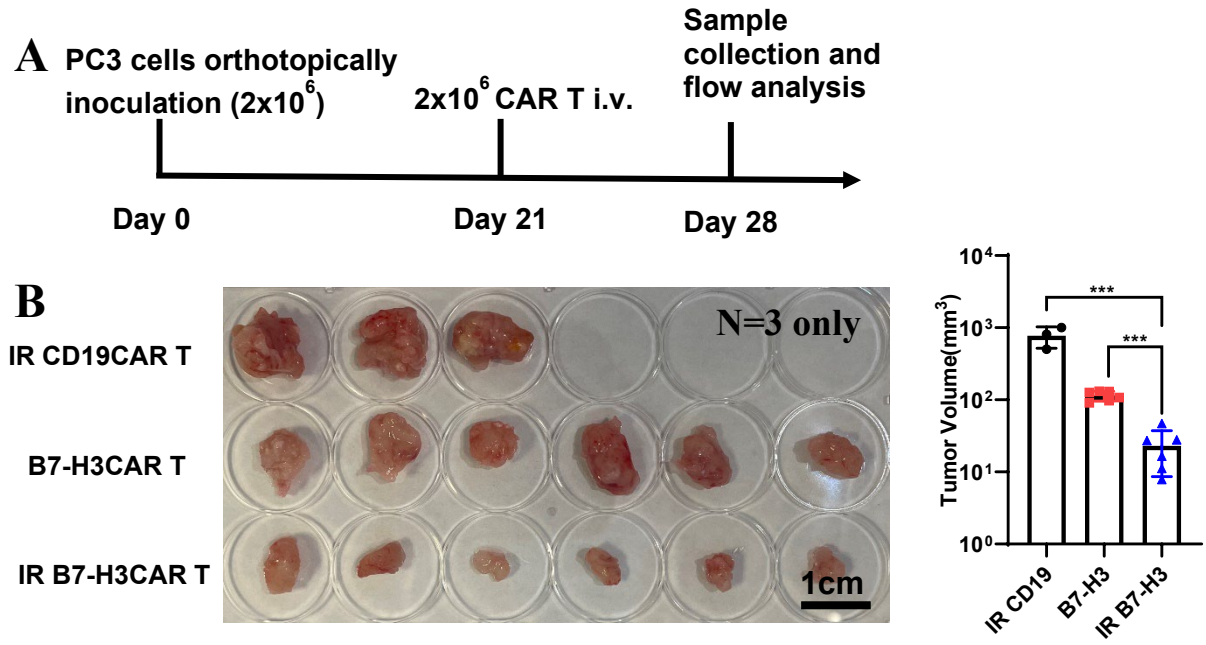

Fig 10. IR ex vivo reprogrammed CAR T cells induced potent anti-CRPC responses in orthotopic human PCRP PCa xenograft growth. Schema of the CRPC orthotopic xenograft model (PC3) and treatment (**A**). Orthotopic tumors collected from each mouse and tumor volumes (mean±SEM) of each group were compared (**B**). *** $p<0.001$.

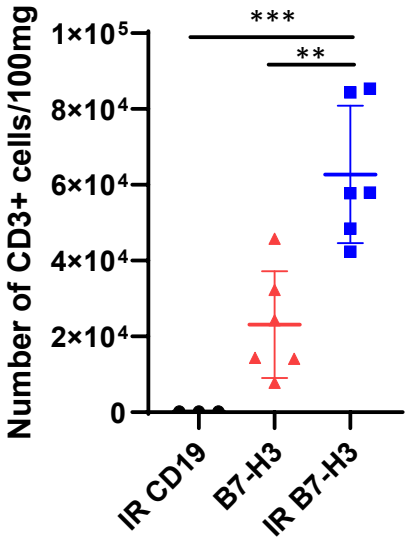

Fig 11. IR ex vivo reprogrammed CAR T cells increased tumor infiltration of CAR T cells in an orthotopic human CRPC PCa xenograft model. Schema of the CRPC orthotopic xenograft model (PC3) and treatment (**Fig10A**). Orthotopic tumors were collected from each mouse (**Fig10B**) and each tumor was digested into a single cell suspension with collagenase IV. Infiltrated CAR T cells, defined as human CD3+ cells, in the digested tumor tissues (100mg) from each group were quantified with counting beads. ** $p<0.01$, *** $p<0.001$.

Aim3: Assess expression frequency and intensity of the B7-H3 epitope recognized by the B7-H3-specific mAb 376.96 used to make the B7-H3 CAR T cells, on PCa cells and PCSCs present in tissue samples from mPCa patients.

Specific aim synopsis

In this aim, we have collected 20 primary, 13 metastatic prostate cancer tissues and 8 cancer-free tissue adjacent to prostate cancer for IHC staining of B7-H3 epitope recognized by monoclonal antibody 376.96. We contacted the Prostate Cancer Biorepository Network (PCBN) for more primary, metastatic prostate cancer tissues, and normal prostate tissues. Unfortunately, the PCBN cannot assist with our specimen request as the PCBC "recently lost our funding from the DoD." To avoid wasting valuable PCa tissues, we are working with the pathology core at MGH using frozen xenograft tissues (B7-H3+) and human tonsil tissues (B7-H3-) to optimize the IHC staining with our homemade mAb 376.96.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Dr. X. Wang and her group at MGH: have trained two graduate students; one was recently awarded Ph.D., and one pre-medical school student was accepted by a medical school in CA, USA.

Dr. Xin Gao at MGH

Dr. Chin-Lee Wu at MGH

Dr. Joseph Schwab at MGH

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

Nothing to report

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We plan to continue the project as proposed based on our current progress.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

More results are to be developed and published. The results obtained thus far look promising to impact upcoming clinical trial design.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

More results are to be developed and published. The results obtained thus far look promising to impact upcoming clinical trial design, such as for other cancer types including leukemia.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. **CHANGES/PROBLEMS:** *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

No significant changes were made.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

There is a delay in performing the specific aim 3. There is a delay in performing the specific aim 3. The Prostate Cancer Biorepository Network (PCBN) no longer provides us with metastatic PCa, primary PCa, and normal prostate tissues.

By working with Dr. Wu, co-I, we can collect enough primary PCa tissue, PCa adjacent cancer-free tissues, and normal prostate tissues.

By working with Drs. Gao and Schwab, co-I, we try to collect enough mPCa tissues. If the number of mPCa tissue is insufficient, we will look for mPCa from commercial vendors.
.

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Nothing to report

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal;*

volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Zhang G, Wang Y, Fuchs BC, Guo W, Drum DL, Erstad DJ, Shi B, [3]Albert AB, Zheng H, Cai L, Zhang L, Tanabe KK, [1] and Wang X. Improving the Therapeutic Efficacy of Sorafenib for Hepatocellular Carcinoma by Repurposing Disulfiram. Front Oncol. 2022; 12: 913736.

PMCID: PMC9329590

Note: We acknowledged this funding for the published work as the 3 of the authors were supported by the funding. Although we used our 'outside working hours," such as weekends, did the analyses and manuscript writing. Without the funding, Wang Y and Drum DL would not have been hired in the first place.

information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**

List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*

- *physical collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*
- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name: Yufeng Wang . MD.

Project Role Graduate student

Nearest person month worked: *7*

Contribution to Project: Dr. Wang performed the in vivo work with Dr Sun and Mr. Drum

Name: Ruochuan Sun. MD.

Project Role Graduate student

Nearest person month worked: *7*

Contribution to Project: Dr. Sun performed the in vitro experiments with Dr. Wang and Mr. Drum

Name: David Drum B.S.

Project Role Technician

Nearest person month worked: *9*

Contribution to Project: Mr Drum performed the in vitro and in vivo experiments with Drs. Wang and Sun.

Name: Feng Chen M. S.

Project Role Graduate student

Nearest person month worked: *3*

Contribution to Project: Ms. Chen has started 3 months ago in the lab and been trained by the PI to perform the proposed experiments.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Nothing to report

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*

None

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.