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CONTRACTING ORGANIZATION: Oregon Health & Science University

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14. ABSTRACT

Enter a brief (approximately 200 words) unclassified summary of the most significant find during the research period.

We investigate a new paradigm for vaccines and immunotherapies of prostate cancer (PCa). We demonstrate that strain 68-1 rhesus cytomegalovirus (RhCMV)-expressing tumor antigens elicit broad CD8+ T cell responses to epitopes presented by non-polymorphic major histocompatibility complex (MHC)-E molecules instead of polymorphic, classical MHC-Ia. Due to the high conservation of MHC-E we were able to show that human PCa cell lines and human primary tumor cells stimulate MHC-E-restricted CD8+ T cells elicited in rhesus macaques (RM) by 68-1 RhCMV expressing prostate acid phosphatase (PAP). We began identifying T cell receptors (TCRs) recognizing PAP-derived peptides presented by MHC-E and characterizing such TCRs. Since MHC-E is non-polymorphic, unlike classical MHC type I molecules, TCRs expressed by MHC-E-restricted

15. SUBJECT TERMS

Immunotherapy of prostate cancer, T cell therapy, major histocompatibility complex E, cancer vaccines using cytomegalovirus vectors

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1. INTRODUCTION:

We propose to investigate a new paradigm for vaccines and immunotherapies of prostate cancer (PCa) that was made possible by our discovery that strain 68-1 rhesus cytomegalovirus (RhCMV)-based vectors elicit broad CD8+ T cell responses to epitopes presented by non-polymorphic major histocompatibility complex (MHC)-E molecules instead of polymorphic, classical MHC-Ia. We will explore the targeting of PCa via prostatic acidic phosphatase (PAP)-specific, MHC-E-restricted CD8+ T cells (ETc) by 1) assessing the capacity of PCa cell lines and primary tumor cells to stimulate ETc, 2) identifying TCRs recognizing PAP-derived peptides presented by MHC-E, 3) transfecting human T cells with these TCRs to identify E-restricted PAP epitopes.

2. KEYWORDS:

Prostate cancer, immunotherapy, human leukocyte antigen E, T cell receptor, prostatic acidic phosphatase, epitope, rhesus macaque, cytomegalovirus, single cell sequencing

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific Aim 1: To assess the capacity of PCa cell lines and primary tumor cells to stimulate PAP-specific, MHC-E-restricted CD8+ T cells.

Task 1 (Month 1): Identification of E-expressing PCa cell lines (Status: completed)

Task 2 (Months 2-3): Monitoring stimulation of ETc by PCa cell lines (Status: completed)

Milestone 1 (Month 3): PCa cell lines identified that stimulate PAP-specific CD8+ T cells via HLA-E (Status: achieved)

Task 3 (Months 1-18): Generating cell suspensions from PAP and E-positive PCa (Status: 50% complete)

Task 4 (Months 1-18): Monitoring stimulation of ETc by primary PCa tumor cells (Status: 50% complete)

Milestone 2 Month 18: HLA-E mediated targeting of PAP-positive PCa tumors demonstrated (Status: partially achieved)

Specific Aim 2: To identify TCRs recognizing PAP-derived peptides presented by PCa cells via MHC-E

Task 5 (Month 4-10): Bulk identification of TCR sequences (Status: completed)

Milestone 3 (Month 10): Frequency of TCRα and TCRβ sequences in PCa reactive and PAP-peptide-specific T cells determined for each RM (Status: completed)

Task 6 (Months 11-18): Identification of individual PAP-specific, MHC-E restricted TCRs (Status: 10% complete)

Milestone 4: Identification of sequences of TCR α/β pairs that were identified in both peptide and PCa-stimulated T cell clones (Status: in progress)

Specific Aim 3: To identify PAP-derived peptides presented by PCa cells via HLA-E

Task 7 (Month 19-36): Mapping of PAP-derived, MHC-E restricted peptides recognized by individual TCR α/β pairs (Status: not started)

Milestone 5 (Month 36): Identification of MHC-E restricted TCRs and PAP epitopes (Status: not started)

Task 8 (Month 19-36): Stimulation of TCR clones by PCa cells (Status: not started)

Milestone 6: Identification of TCRs recognizing cancer cell lines and primary cancer cells (Status: not started)

Milestone 7: MHC-E restricted PAP epitopes shared by, or limited to, individual tumors (Status: not started)

Major Activity 1: To assess the capacity of PCa cell lines and primary tumor cells to stimulate PAP-specific, MHC-E-restricted CD8+ T cells.

Specific Objective 1.1: Expression of HLA-E on established PCa tumor cell lines

We obtained the following PAP-expressing prostate cancer cell lines from ATCC: LNCaP, 22RV1, VCaP, PC3, DU145 and NCI-H660. In addition, the Mt Sinai team generated androgen-resistant sub-clones of LNCaP that were tested. We also generated K562 cells (that lack endogenous HLA-ABC and express low levels of HLA-E) stably expressing HLA-E alone or together with full-length PAP or truncated PAP missing the C-terminal third or half of the protein. The cell lines were cultured according to published protocols and HLA-E expression was monitored by immunoblot of cell lysates. Immunoblots were performed by re-suspending cells in SDS lysis buffer followed by SDS gel-electrophoresis, electrophoretic transfer onto membranes and probing with HLA-E-specific antibodies 3D12 (2) or 4D12 (3). As summarized in **Table 1**, we were able to detect HLA-E in all prostate cancer cell lines tested albeit at different levels. PAP expression has previously been shown for LNCaP, VCaP and 22Rv1 whereas it is absent from DU145 and PC-3 cells (4-7). mRNA levels for PAP from HC660 and other cell lines shown in table 1 are based on published information from the Broad-Institute (1).

Prostate Cancer Cells	HLA-E	PAP
LNCaP (Parental)	+	+++
LNCaP clone A	+	+++
LNCaP clone 2	+	+++
LNCaP clone 8	+	+++
22RV1	+	++
VCaP	+++	+++
PC3	++	-
DU145	+++	-
NCI-H660	+	+
K562 Controls:		
K562	+	-
K562-E	+++	-
K562-E-PAP	+++	+++
K562-E-PAP truncated 1/3	+++	+++
K562-E-PAP truncated 1/2	+++	+++

Table 1: HLA-E expression in prostate cancer cell lines. Shown is the expression of HLA-E as observed in immunoblots. Expression levels were compared to PAP-transfected K562 cells: +++ similar or better expression compared to K562 cells, ++ weaker expression, + expression only detectable upon prolonged exposure. Expression levels for PAP were either determined by immunoblot (PAP-transfected K562 cells and LNCaP clones compared to LNCaP cells) or are based on RNA expression as determined by RNAseq as part of the Broad Institute cancer cell line encyclopedia (1). Gene expression levels above 4 are shown as +++, above 1 = ++, above 0 = + and below 0 = -

Specific Objective 1.2: Monitoring the stimulation of MHC-E-restricted, PAP-specific CD8+ T cells by prostate cancer cell lines.

We determined whether PAP-expressing prostate cancer cell lines would be able to stimulate CD8+ T cells isolated from peripheral blood mononuclear cells from rhesus macaques (RM) immunized with strain 68-1 RhCMV expressing rhesus PAP (RhPAP). After enrichment by magnetic bead sorting, CD8+ T cells were co-incubated with PCa cell lines and T cell stimulation was monitored by intracellular cytokine staining (ICS) for interferon-gamma (IFNγ) and tumor necrosis factor alpha (TNFα). For the cancer cell lines LNCaP, VCaP and 22RV1 we observed robust production of TNΦα and IFNγ by CD8+ T cells from RhCMV/PAP vaccinated RMs whereas PC3 was negative (**Fig. 1A**). This response was specific for PAP as demonstrated by the fact that HLA-E expressing K562 cells (K562-E) did not stimulate these T cells unless also transfected with RhPAP (K562-E PAP). Importantly, T cell stimulation was abolished by adding the HLA-E ligand peptide VL9 thus demonstrating that these cancer cell lines presented PAP-derived peptides specific CD8+ T cells recognized these cancer cell lines (**Fig. 1B**).

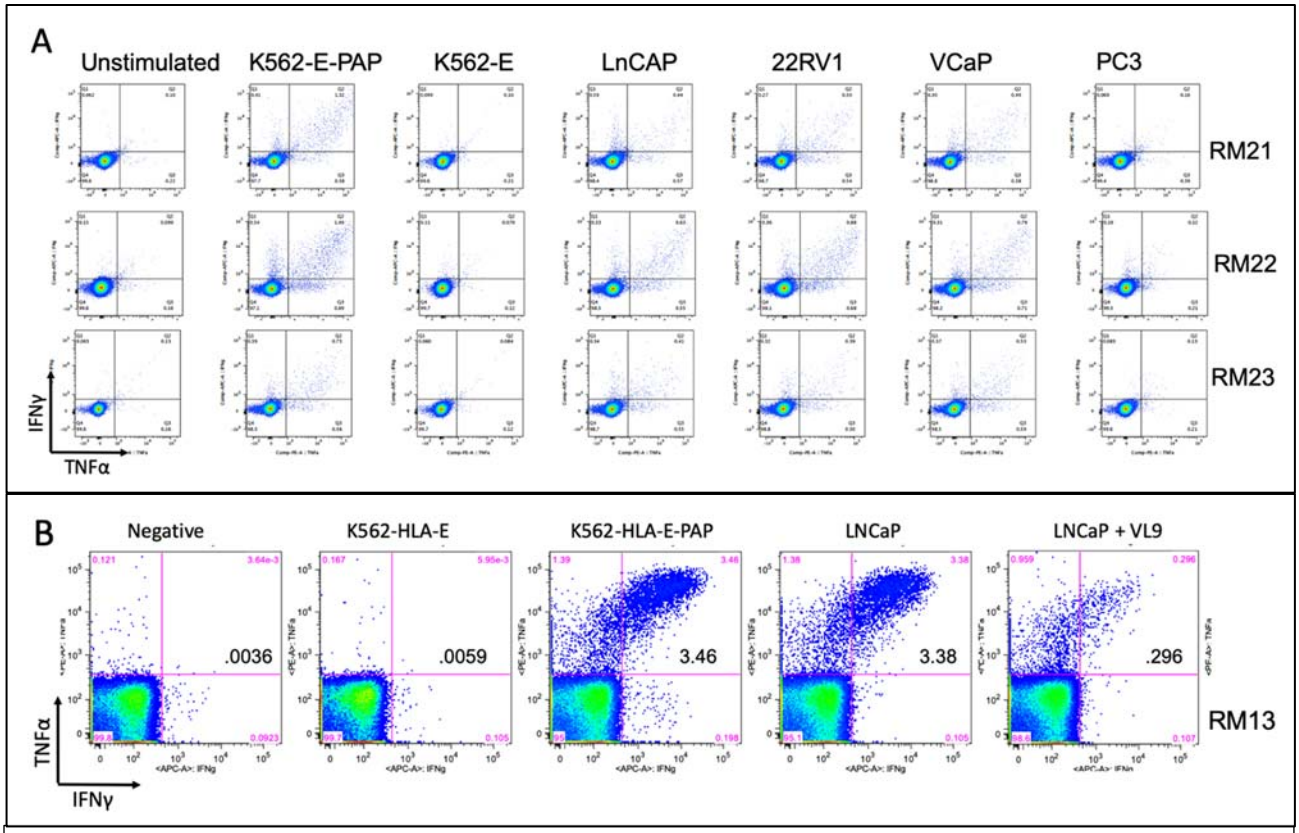

Figure 1: CD8+ T cells from RhCMV/RhPAP-immunized RM recognize PAP-expressing cancer cell lines.
A) CD8+ T cells were isolated from three RM previously immunized with 68-1 RhCMV/RhPAP and co-incubated with indicated cell lines. Dot plots showing the results of ICS for IFNγ and TNFα staining for each cell line.
B) CD8+ T cells were co-incubated with the indicated cell lines as in A) except that peptide VL9 was added prior to and during ICS where indicated.

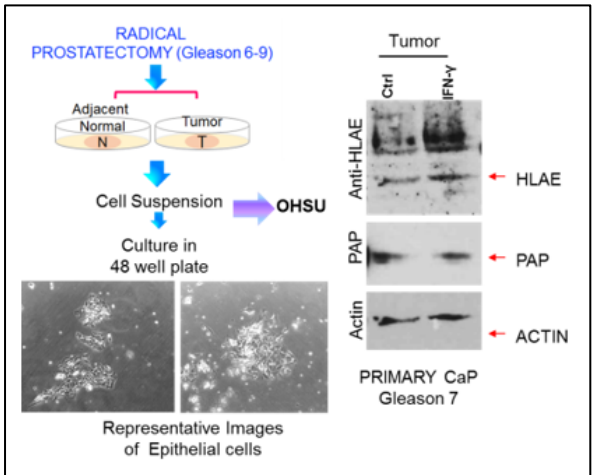

Figure 2: HLA-E and PAP expression in prostate cancer cell suspension. Left: schematic of cell suspension and picture of epithelial cell clusters. Right: immunoblot of tumor cells in the presence or absence of IFNγ.

Specific Objective 1.3: Generating cell suspensions from PAP and HLA-E-positive prostate cancer samples (objective 1.3 was conducted by our subcontractor Mt. Sinai)

Isolation of viable cells from radical prostatectomy (surgical resection) tissue specimens: Explant cultures and/or cell suspensions from primary prostate cancer tissues (Gleason's 6-10) were generated from prostate tissue of subjects consented to PPHS/IRB study (Dr. Ash Tewari Mount Sinai # GCO 14-0318) as described earlier (8-13) with modifications. At tissue grossing ~4mm^{3}tissues corresponding to adjacent normal and/or tumor area were freshly dissected by resident pathologist and de-identified tissue specimens were transported in MACS tissue storage solution (Miltenyi Biotech) supplemented with 10µM Rock Inhibitor (Selleck Chemicals), to the laboratory. Tissues were washed

twice in DMEM and minced into smaller 1mm^3 pieces. Cell suspensions were prepared using tumor dissociation kit (Miltenyi Biotech) and gentle-MACS dissociator (program 37C_h_TDK_2) for 60 min. The cell suspension was filtered using a 70µm strainer and collected by centrifugation at 430g for 10 min. Cells were suspended in culture media (MEM supplemented with 5%FBS, 0.01 mg/ml insulin and 25 µmol/L hydrocortisone (Sigma), and 1x pen/strep and plated in 24 well plates at 1x10^4 cells/well. Primary prostate epithelial clones develop in 2 days and were used for further studies. (**Fig 2**). Suspension cells developed using this method consist of a heterogeneous mix of prostate epithelial cells, stromal cells and tissue resident immune cells and were frozen in Cyrostor solution (Stemcell Technologies). Cell suspensions from prostate cancer tissue of 10 different patients were shipped to OHSU.

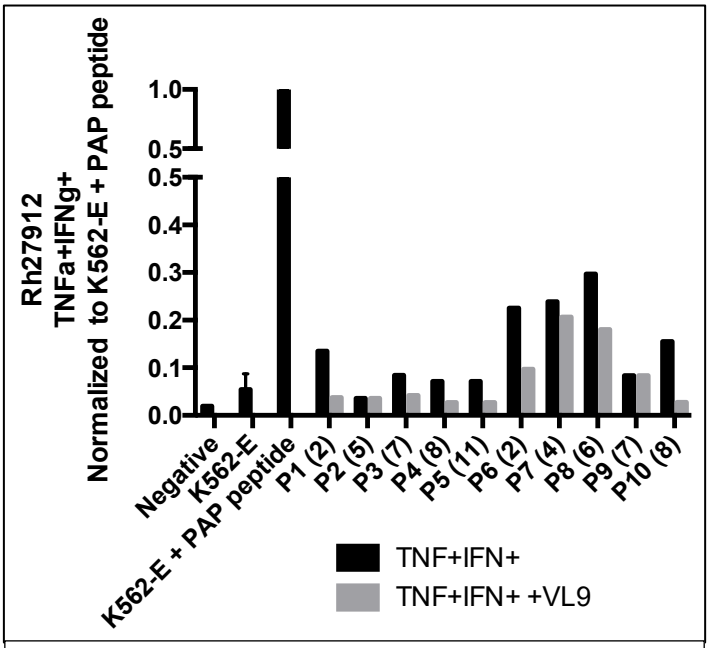

Figure 3: MHC-E-restricted CD8+ T cells from RhCMV/RhPAP-immunized RM are stimulated by primary tumor cell. CD8+ T cells were isolated from RM 11 (Rh27912) previously immunized with 68-1 RhCMV/ RhPAP and co-incubated with cell suspensions obtained from recently resected prostate cancer tissue from 10 patients (P1-10). For control we used K562-E cells in the presence or absence of RhPAP peptide pools. To eliminate MHC-II stimulation we added anti-DR and CLIP to all stimulations. Where indicated we added MHC-E binding peptide VL9 to demonstrate HLA-E dependent peptide presentation. The graph shows the percent CD8+ T cells generating IFNγ or TNFα upon co-incubation with cancer cells.

Specific Objective 1.4: Monitoring the stimulation of MHC-E-restricted, PAP-specific CD8+ T cells by primary tumor cells

Upon arrival at OHSU, prostate cancer cell suspensions of 10 different patients (P1-P10) were co-incubated with CD8+ T cells from RM immunized with 68-1 RhCMV/RhPAP in the presence or absence of MHC-E-specific peptide VL9. To avoid stimulating MHC-II-restricted CD8+ T cells we also added MHC-II binding peptide CLIP and anti-DR antibody to all stimulations. For positive control, we added a pool of 15mer peptides that spanned the entire RhPAP sequence and overlapped by 11AA. As shown in **Figure 3**, the CD8+ T cells from RM showed above-background staining for TNFα or IFNγ in the presence of prostate cancer cells from several patients. Since in most instances this staining was reduced in the presence of MHC-E binding peptide VL9, we conclude that prostate cancer cells present PAP antigen via HLA-E.

<u>Conclusion for Major Activity 1:</u> Our data suggest that prostate cancer cells are able to present peptides derived from PAP via the conserved HLA-I molecule HLA-E. These results strongly support the notion that prostate cancer can be targeted by HLA-E restricted CD8+ T cells.

Major Activity 2: To identify TCRs recognizing PAP-derived peptides presented by PCa cells via MHC-E

Objective 2.1 Bulk identification of TCR sequences

To identify individual T cell receptors expressed by MHC-E restricted, PAP-specific CD8+ T cells we co-incubated CD8+ T cells isolated from RM immunized previously with 68-1 RhCMV/RhPAP with K562-E cells expressing RhPAP. For internal control, animals were also immunized with 68-1 RhCMV expressing the gag protein of simian immunodeficiency virus (SIV). To enable FACS sorting of the responsive T cells we performed T cell simulation in the presence of the compound TAPI-0, a metalloprotease inhibitor which prevents cleavage of TNFα and therefore traps it on the surface of activated cells. We then sorted activated, CD69 and TNFα positive, CD8+ T cells, which were processed using the 10x Genomics scRNA-seq system. This produces Illumina-compatible sequencing libraries, which have been enriched by PCR for near full-length TCR molecules. Molecules from each individual cell are tagged with a molecular barcode unique to that cell. As shown in **Fig. 4** for the TCRβ-chain of one of the animals, this sequencing revealed the CDR3 clonotypic hierarchy for each PAP and SIVgag, with 3-4 major clones per antigen. TCRβ CDR3 is shown by itself for simplicity; however, the 10x genomic system captures near-full length TCR sequence and critically it also captures the paired TCRα sequence for each clone.

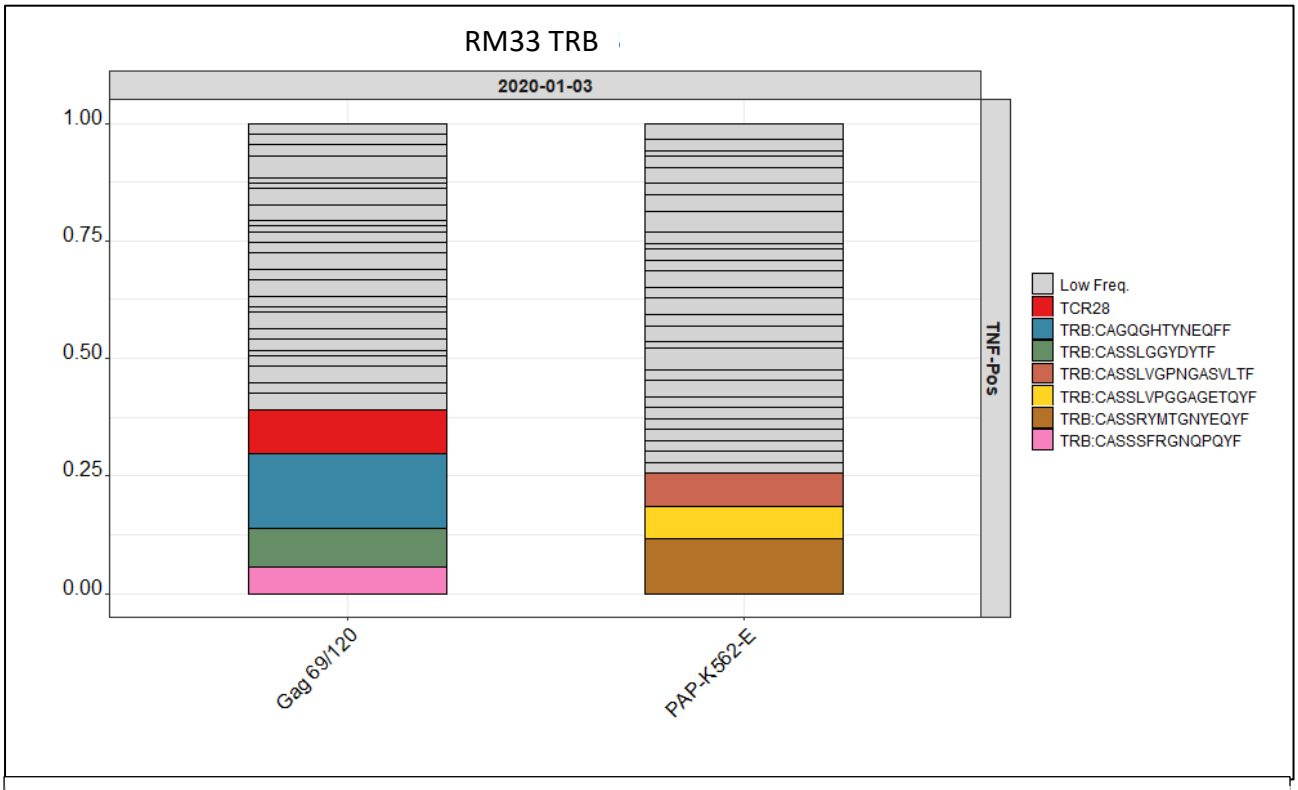

Figure 4: TCR clonotypic hierarchies of SIVgag and PAP-specific CD8+ T cells. RM33 had been previously immunized with 68-1 RhCMV/RhPAP and 68-1 RhCMV/SIVgag. Shown are the relative frequencies of individual TCRβ sequences observed CD8+ T cells obtained stimulated with with SIVgag-derived, MHC-E restricted peptides Gag69 and Gag120 or with K562 cells expressing RhPAP. The sequences of the complementarity determining region 3 (CDR3) of the most frequent TCRs are shown on the right. TCR28 has been previously found in SIVgag-specific responses in other RM thus validating the technique.

Objective 2.2 Identification of individual PAP-specific, MHC-E restricted TCRs

Based on these results, we selected 2 specific TCR αβ pairs for validation and further analysis. The respective TCRs were synthesized as a single, bicistronic ORF expressing full length α and β TCR sequences (separated by a P2A cleavage site) with the TCR constant regions replaced with that of murine orthologs. These hybrid TCRs display increased cell surface density by avoiding incorrect pairing with the endogenous αβTCR chains, they improve stability of the TCR/CD3 complex thus enhancing T cell signaling and functionality. The synthetic genes were inserted into the retroviral expressing vectors. As shown in **Fig. 5**, both TCRs were expressed on the surface of the retroviral packaging cell line.

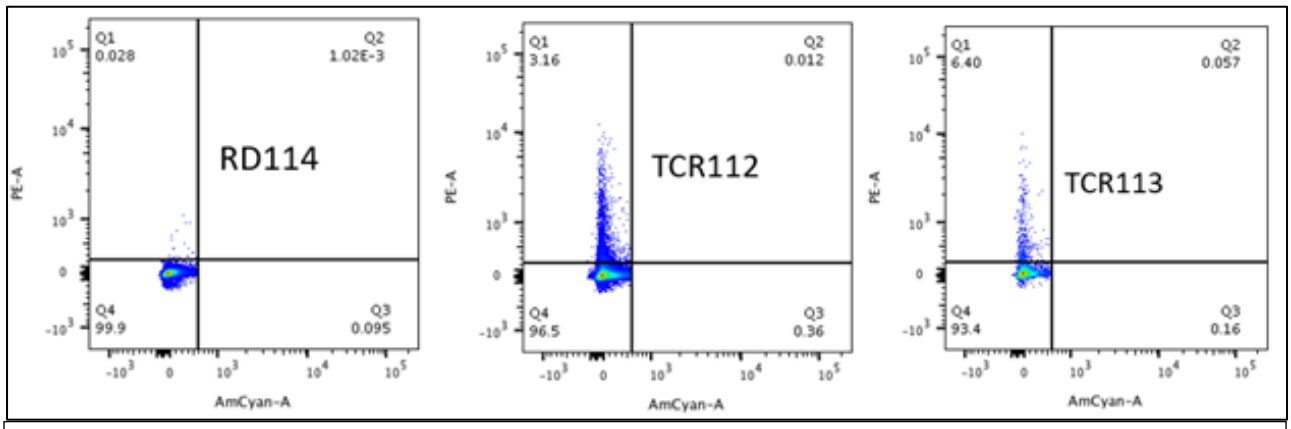

Figure 5: Expression of cloned TCRs isolated from RhCMV/PAP immunized RM. The RD114 retroviral packaging cell line was transfected with plasmids encoding recombinant TCRs (TCR112 and TCR113 panels) or mock transfected (RD114). After 48 hours, retroviral vectors were harvested from the supernatants. The transfected cells were surface stained for murine TCR β constant region expression and evaluated by flow cytometry.

Conclusion for Major Activity 2: We have established the methodology to identify and clone TCRs from MHC-E-restricted, PAP-specific CD8+ T cells.

References cited:

1. Ghandi M, Huang FW, Jané-Valbuena J, Kryukov GV, Lo CC, McDonald ER, Barretina J, Gelfand ET, Bielski CM, Li H, Hu K, Andreev-Drakhlin AY, Kim J, Hess JM, Haas BJ, Aguet F, Weir BA, Rothberg MV, Paolella BR, Lawrence MS, Akbani R, Lu Y, Tiv HL, Gokhale PC, de Weck A, Mansour AA, Oh C, Shih J, Hadi K, Rosen Y, Bistline J, Venkatesan K, Reddy A, Sonkin D, Liu M, Lehar J, Korn JM, Porter DA, Jones MD, Golji J, Caponigro G, Taylor JE, Dunning CM, Creech AL, Warren AC, McFarland JM, Zamanighomi M, Kauffmann A, Stransky N, Imielinski M, et al. 2019. Next-generation characterization of the Cancer Cell Line Encyclopedia. Nature 569:503-508.
2. Lee N, Goodlett DR, Ishitani A, Marquardt H, Geraghty DE. 1998. HLA-E surface expression depends on binding of TAP-dependent peptides derived from certain HLA class I signal sequences. J Immunol 160:4951-60.
3. Ishitani A, Sageshima N, Lee N, Dorofeeva N, Hatake K, Marquardt H, Geraghty DE. 2003. Protein expression and peptide binding suggest unique and interacting functional roles for HLA-E, F, and G in maternal-placental immune recognition. J Immunol 171:1376-84.

4. Johnson IR, Parkinson-Lawrence EJ, Butler LM, Brooks DA. 2014. Prostate cell lines as models for biomarker discovery: performance of current markers and the search for new biomarkers. Prostate 74:547-60.
5. Quintero IB, Araujo CL, Pulkka AE, Wirkkala RS, Herrala AM, Eskelinen EL, Jokitalo E, Hellstrom PA, Tuominen HJ, Hirvikoski PP, Vihko PT. 2007. Prostatic acid phosphatase is not a prostate specific target. Cancer Res 67:6549-54.
6. Lin MF, Meng TC, Rao PS, Chang C, Schonthal AH, Lin FF. 1998. Expression of human prostatic acid phosphatase correlates with androgen-stimulated cell proliferation in prostate cancer cell lines. J Biol Chem 273:5939-47.
7. Korenchuk S, Lehr JE, L MC, Lee YG, Whitney S, Vessella R, Lin DL, Pienta KJ. 2001. VCaP, a cell-based model system of human prostate cancer. In Vivo 15:163-8.
8. Goodwin JF, Kothari V, Drake JM, Zhao S, Dylgjeri E, Dean JL, Schiewer MJ, McNair C, Jones JK, Aytes A, Magee MS, Snook AE, Zhu Z, Den RB, Birbe RC, Gomella LG, Graham NA, Vashisht AA, Wohlschlegel JA, Graeber TG, Karnes RJ, Takhar M, Davicioni E, Tomlins SA, Abate-Shen C, Sharifi N, Witte ON, Feng FY, Knudsen KE. 2015. DNA-PKcs-Mediated Transcriptional Regulation Drives Prostate Cancer Progression and Metastasis. Cancer Cell 28:97-113.
9. Schiewer MJ, Goodwin JF, Han S, Brenner JC, Augello MA, Dean JL, Liu F, Planck JL, Ravindranathan P, Chinnaiyan AM, McCue P, Gomella LG, Raj GV, Dicker AP, Brody JR, Pascal JM, Centenera MM, Butler LM, Tilley WD, Feng FY, Knudsen KE. 2012. Dual roles of PARP-1 promote cancer growth and progression. Cancer Discov 2:1134-49.
10. Centenera MM, Raj GV, Knudsen KE, Tilley WD, Butler LM. 2013. Ex vivo culture of human prostate tissue and drug development. Nat Rev Urol 10:483-7.
11. Maranto C, Udhane V, Hoang DT, Gu L, Alexeev V, Malas K, Cardenas K, Brody JR, Rodeck U, Bergom C, Iczkowski KA, Jacobsohn K, See W, Schmitt SM, Nevalainen MT. 2018. STAT5A/B Blockade Sensitizes Prostate Cancer to Radiation through Inhibition of RAD51 and DNA Repair. Clin Cancer Res 24:1917-1931.
12. Drost J, Karthaus WR, Gao D, Driehuis E, Sawyers CL, Chen Y, Clevers H. 2016. Organoid culture systems for prostate epithelial and cancer tissue. Nat Protoc 11:347-58.
13. Karthaus WR, Iaquinta PJ, Drost J, Gracanin A, van Boxtel R, Wongvipat J, Dowling CM, Gao D, Begthel H, Sachs N, Vries RGJ, Cuppen E, Chen Y, Sawyers CL, Clevers HC. 2014. Identification of multipotent luminal progenitor cells in human prostate organoid cultures. Cell 159:163-175.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

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

We will clone additional TCR sequences from PAP-specific, MHC-E restricted CD8+ T cells. We will transfect these TCRs into primary T cells. We will determine the stimulation of these cloned TCRs by PAP expressing cancer cells. We will identify individual peptides stimulating these TCRs.
We also plan to publish a paper detailing our findings.

4. **IMPACT:**
What was the impact on the development of the principal discipline(s) of the project?

We show for the first time that cancer cells not only express HLA-E, but can stimulate CD8+ T cells that recognize the tumor antigen PAP via HLA-E. This finding suggests that HLA-E presents tumor antigen-derived peptides on cancer cells thus enabling the targeting of HLA-E by immunotherapy. Since HLA-E is a non-polymorphic HLA molecule, this finding will enable the development of immunotherapies that are universal, i.e. they will work regardless of the HLA-type of a patient. We began to isolate and characterize T cell receptors (TCRs) specific for HLA-E presenting cancer antigens to enable immunotherapy by transgenic T cells expressing such TCRs.

What was the impact on other disciplines?

The techniques applied and optimized in this proposal will be generally applicable to isolate and characterize T cell receptors that recognize peptides in the context of HLA-E.

What was the impact on technology transfer?

The results of this work have been included in a patent application that was licensed to Vir Biotechnology.

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

A limitation of studying primary tumor cells was the limited cell numbers available from resected prostate cancer. We will mitigate this problem in two ways: a) we will try to isolate more cells from large prostate tumors, b) we will use PAP-expressing acute myelogenic leukemia cells (AML) to confirm some of the key results since there is no shortage of AML cells.

Changes that had a significant impact on expenditures

Nothing to report

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

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS

- **Publications, conference papers, and presentations**
  Journal publications.

  Nothing to report

  Books or other non-periodical, one-time publications.

  Nothing to report

  Other publications, conference papers and presentations.

  Nothing to report

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

  Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Patent application PCT/US2020/036481 entitled "Tumor Antigen-specific T cell responses" was submitted by OHSU based on provisional application No. 62/878,511, filed July 25, 2019, which claims the benefit of U.S. Provisional Application No. 62/858,756, filed June 7, 2019. Inventors are Klaus Frueh, Scott Hansen and Louis Picker. The patent was licensed exclusively to Vir Biotechnology as an appendix to a master licensing agreement between OHSU and Vir.

- **Other Products**

Sequences for TCR112 and TCR113 will be provided to the public in publications if studies confirm their specificity for PAP-peptides presented by MHC-E

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

VACCINE & GENE THERAPY INSTITUTE/OHSU

Name:	Klaus Frueh
Project Role:	Principal Investigator
NIH Commons ID:	kfrueh
Nearest person month worked:	1
Contribution to Project:	As the Principal Investigator of the project, Dr. Früh supervised the experiments and interpreted the data together with other investigators on the project.

Name:	Ben Bimber
Project Role:	Co-Investigator
NIH Commons ID:	bimberb
Nearest person month worked:	1
Contribution to Project:	Dr. Bimber performed the bioinformatics analysis of sequencing results that identified the frequency MHC-E-restricted, PAP-specific TCRs. He helped selecting individual TCR constructs.

Name: Scott G. Hansen
Project Role: Co-Investigator
NIH Commons ID: hansensc
Nearest person month worked: 1
Contribution to Project: Dr. Hansen supervised the T cell assays with monkey-derived T cells using cancer cells or PAP-derived peptides.

Name: Louis Picker
Project Role: Co-Investigator
NIH Commons ID: pickerl
Nearest person month worked: 1
Contribution to Project: Dr. Picker advised Drs Frueh and Hansen with respect to experimental design, data analysis and trouble shooting.

Name: Kyle J. Taylor
Project Role: Research Assistant 2
Researcher Identifier (e.g. ORCID ID): not applicable
Nearest person month worked: 2
Contribution to Project: Ms. Taylor assisted Dr. Bimber with sequence analysis of PAP-specific TCRs.

Name: Abigail B. Ventura
Project Role: Research Associate
Researcher Identifier (e.g. ORCID ID): not applicable
Nearest person month worked: 2
Contribution to Project: Ms. Venture assisted Dr. Hansen with processing samples for T cell assays. She also assisted Dr. Bimber by FACS sorting of single MHC-E restricted T cells specific for PAP.

MT. SINAI SCHOOL OF MEDICINE

Name: Ashutosh Kumar Tewari
Project Role: Subaward Principal Investigator
NIH Commons ID: aktewari
Nearest person month worked: 1
Contribution to Project: Dr. Tewari provided prostate cancer samples for the project and coordinated the collaboration between Mt Sinai and OHSU. He arranged for regular meetings between the two teams and helped with data analysis in interpretatoin

Name: Nina Bhardwaj
Project Role: Subaward Co-Investigator
NIH Commons ID: bhardno2
Nearest person month worked: 1
Contribution to Project: Dr. Bhardwaj worked with Drs Tewari and Nair in designing the experiments using primary prostate cancer samples for immunological studies.

Name:	Sujit Nair
Project Role:	Subaward Co-Investigator
NIH Commons ID:	nairs1
Nearest person month worked:	1
Contribution to Project:	Dr. Nair isolated and cultured prostate cancer cells and provided them to OHSU for T cell analysis.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Vir Biotechnology Inc. located in San Francisco provided funding for the animal cohorts used in this research. The Bill and Melinda Gates foundation, located in Seattle, and NIAID, located in Bethesda, provided funding for the development of tools such as TCR sequencing used in this project. Principal investigators at the Vaccine and Gene Therapy Institute (VGTI) of OHSU provided advice and input in experimental design and interpretation of the results.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHARTS: Attached

9. APPENDICES: N/A