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14. ABSTRACT

In this reporting period, we developed an in vitro method to reprogram prostate adenocarcinoma to neuroendocrine prostate cancer (NEPC) which has enabled unprecedented functional characterization of candidate factors involved in NE transdifferentiation. We also expanded our characterization of CEACAM5 expression in lethal metastatic castration-resistant prostate cancer relative to other cell surface antigens under clinical investigation. We redirected the CEACAM5 antibody-drug conjugate labetuzumab govitecan originally developed for colorectal cancer to prostate cancer and demonstrate striking antitumor activity across multiple preclinical models of CEACAM5^{+} NEPC. Ongoing studies with an L1CAM CE7 chimeric antigen receptor also indicate antigen-specific T cell activation and cytotoxicity against NEPC cell lines. Lastly, we have started to evaluate six fully humanized L1CAM CE7 candidate antibodies for specific binding to the tumor-selective, glycosylated CE7 epitope.

15. SUBJECT TERMS
Neuroendocrine prostate cancer, cell surface antigens, antibody therapy, immunotherapy

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INTRODUCTION

Background: Neuroendocrine prostate cancer (NEPC) is a common, deadly endpoint for men with late-stage metastatic prostate cancer. Up to 20% of men with lethal metastatic prostate cancers demonstrate evidence of NEPC [1, 2]. NEPC can be distinguished from conventional prostate cancer or prostate adenocarcinoma (PrAd) by histologic features, neuroendocrine (NE) marker expression, loss of either androgen receptor (AR) or downstream AR signaling, Polycomb repressive complex expression, and global methylation patterns [3-5]. Genomic studies of NEPC have also identified frequent amplification of *MYCN* and loss of both *TP53* and *RB1* [5, 6]. NEPC represents a cancer differentiation state distinct from PrAd and we hypothesize that the cell surface phenotype of these prostate cancer subtypes should reflect these differences. Furthermore, these differences in cell surface antigen expression may provide an opportunity for prostate cancer subtype-specific therapeutic targeting. The purpose of the research is to establish new NEPC models, to characterize the differential cell surface antigen profile of NEPC relative to PrAd, and to develop antibody reagents targeting novel antigens in NEPC. We believe that these studies may facilitate the development of targeted treatments for men with NEPC, for which there are no currently available FDA-approved therapeutics.

KEYWORDS

Neuroendocrine prostate cancer, cell surface antigens, antibody therapy, immunotherapy

ACCOMPLISHMENTS

To summarize the research accomplishments to date, the tasks described in the proposed Statement of Work are itemized here with a brief update for each task.

SA 1: Establish a diverse panel of NEPC cell lines from human prostate epithelial transformation (months 1-12)

Task 1: Use the human prostate organoid transformation assay with the oncogenes MYCN and activated AKT1 to generate new NEPC cell lines (months 1-12) Completed during prior reporting periods.

Task 2: Evaluate the effect of p53 loss and Rb loss in combination with the oncogenes MYCN and activated AKT1 in initiation of NEPC (months 1-24) Completed. The initial focus of this task was to generate NEPC models from the transformation of benign basal prostate epithelial cells. However, we reasoned that NEPC most often arises from conventional PrAd under the selective pressure of treatment and therefore a model of lineage reprogramming from androgen receptor (AR) pathway-active prostate cancer (ARPC) to NEPC would be more biologically relevant to human disease. To address this issue, we used the C4-2B and LNCaP ARPC cell lines and adopted a cellular reprogramming approach akin to that used by Dr. Shinya Yamanaka's group to determine the critical factors necessary to established induced pluripotency [7]. We introduced a rationally selected candidate factor lentiviral pool composed of mutant p53 R175H, a short-hairpin targeting Rb1 (shRb1), N-Myc, and other factors associated with NEPC (Fig. 1A). Of note, myristoylated AKT1 was not added to the pool because the C4-2B and LNCaP cell lines already demonstrate AKT activation due to *PTEN* loss. Each lentivirus was introduced at a multiplicity-of-infection of two for each cell line and, after three days, placed into neural stem cell media (N-SCM) which supports the propagation of NEPC cell lines. Cells were subsequently collected on day 14 for phenotypic analysis by immunoblot analysis, embedded as cell clusters in Histogel for immunohistochemical (IHC) analysis, or injected subcutaneously into immune-deficient male, non-castrate NSG mice for assessment of tumor initiation and histology.

We discovered that the candidate factor lentiviral pool could indeed lead to a substantial reduction in AR and AR-dependent NK3 homeobox 1 (NKX3-1) protein expression while simultaneously inducing the expression of multiple established markers of NE differentiation including forkhead box A2 (FOXA2), synaptophysin (SYP), neural cell adhesion molecule 1 (NCAM1), and insulinoma-associated protein 1 (INSM1) in the C4-2B and LNCaP cell lines (Fig. 1B). Loss of nuclear AR expression

and gain of SYP expression was evident in the Histogel-embedded cell clusters as well as the resultant tumor arising after xenotransplantation (Fig. 1C-D). RNA-seq gene expression analysis of the cell line models using established gene signatures showed significant enrichment of a NE signature and de-enrichment of an AR signature (Fig. 1E). Further, *AR* and *NKX3-1* were within the most down-regulated nuclear hormone receptors and transcriptions factors relative to controls (Fig. 1F). These results indicate the successful development of an *in vitro* NE transdifferentiation assay that recapitulates NE lineage reprogramming of prostate cancer in a controlled manner.

We next sought to determine the functional contribution of each candidate factor to the pool in reprogramming from ARPC to NEPC. We performed the NE transdifferentiation assay with a leave-one (factor)-out approach, in which each factor was left out from the pool with the readout being comparative analysis of AR and NE marker expression (Fig. 2A). The most striking finding was that the exclusion of ASCL1 from the pool rescued AR and NKX3-1 expression and abrogated FOXA2, NCAM1, and INSM1. We also employed a factor reconstitution approach to further validate functional effects on AR and NE programs in the ARPC cell lines. We excluded NR0B2 and KRAS G12V because of their apparently minimal contributions to the candidate factor lentiviral pool in the leave-one-out analysis. ASCL1 expression

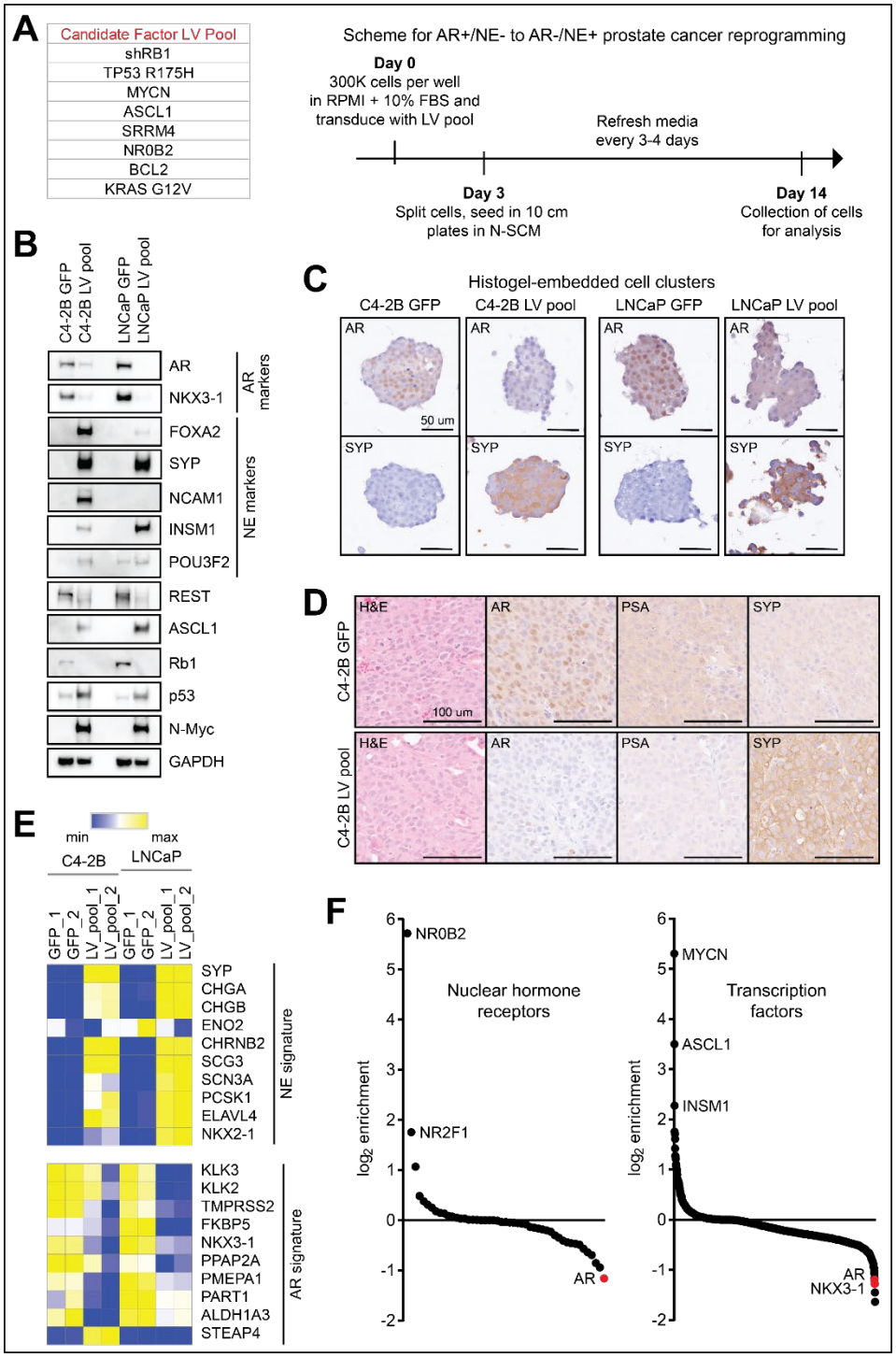


Fig. 1. Direct reprogramming of ARPC to NEPC. (**A**) Candidate factor LV pool and scheme for *in vitro* prostate cancer lineage reprogramming. (**B**) Immunoblot and (**C**) immunocytochemical analysis of the respective C4-2B and LNCaP lines at day 14. (**D**) Photomicrographs of stained tissue sections of C4-2B cell line xenografts established in non-castrate adult male NSG mice. (**E**) RNA-seq gene expression heatmap showing 10-gene NE and AR signatures for the C4-2B and LNCaP lines modified with GFP or LV pool. (**F**) Plot showing ranked enrichment of nuclear hormone receptor (*left*) or transcription factor (*right*) gene expression in the LV pool relative to GFP conditions.

could downregulate AR and NKX3-1 and induce NE markers (Fig. 2B). These results implicate ASCL1 as a critical player in NE transdifferentiation of prostate cancer.

Interestingly, introduction of p53 R175H, shRB1, N-Myc and Bcl-2 were insufficient to enforce NE transdifferentiation. This was consistent with these factors not being critical to NE lineage reprogramming in the leave-one-out analysis and recently published work indicating that NE transdifferentiation is not an obligate outcome of p53 and Rb1 loss in prostate cancer [8]. Given that alterations in *TP53*, *RB1*, *MYCN*, and *BCL2* are common in NEPC, we questioned why these factors appeared dispensable in our studies. We rationalized that the survival of a new cancer state arising from lineage reprogramming would require the acquisition of competitive cell fitness. We hypothesized that alterations in these genes may enhance cell fitness through established effects on cell cycle dysregulation and inhibition of apoptosis. Indeed, we found that deregulation of *TP53*, *RB1*, *MYCN*, and *BCL2* in addition to overexpression of the splicing factor *SRRM4* enhanced proliferation and overcame the negative impact of ASCL1 on proliferation (Fig. 2C). Our findings highlight competing forces of cellular differentiation and proliferation and suggest a functional hierarchy of drivers and facultative factors during NE lineage reprogramming of prostate cancer.

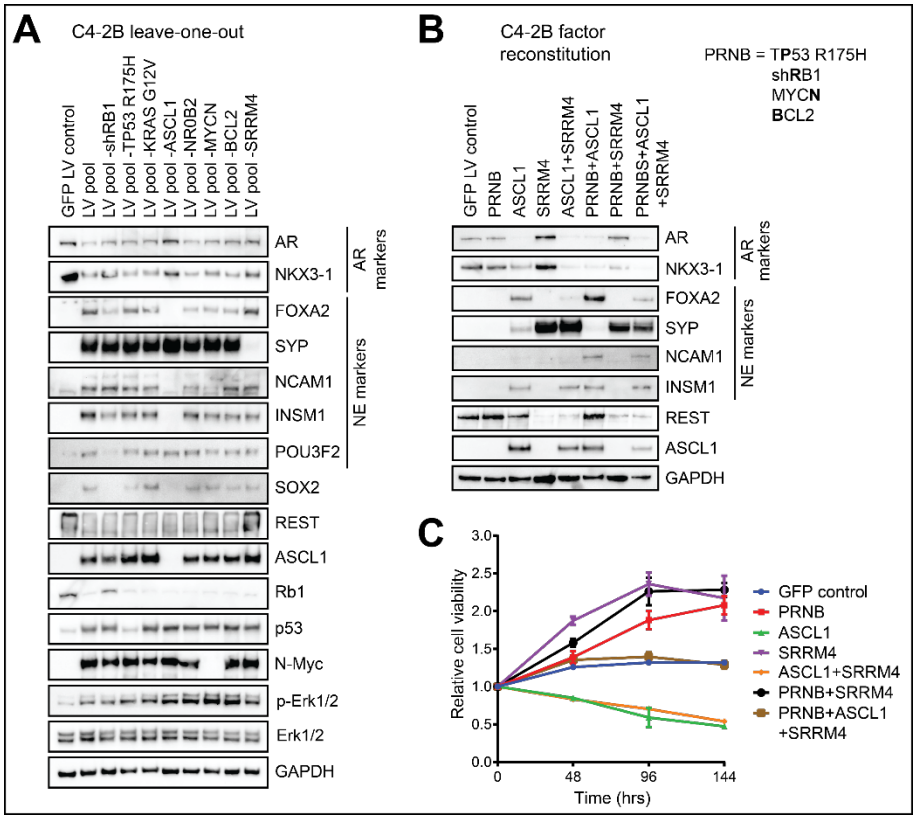


Fig. 2. The pioneer neural transcription factor ASCL1 drives NE transdifferentiation. Immunoblot analysis of (**A**) leave-one-out and (**B**) factor reconstitution conditions in C4-2B reprogramming studies. (**C**) Relative cell viability over time of C4-2B lines modified with various factors and factor combinations. Similar results in the LNCaP cell line are not shown for brevity.

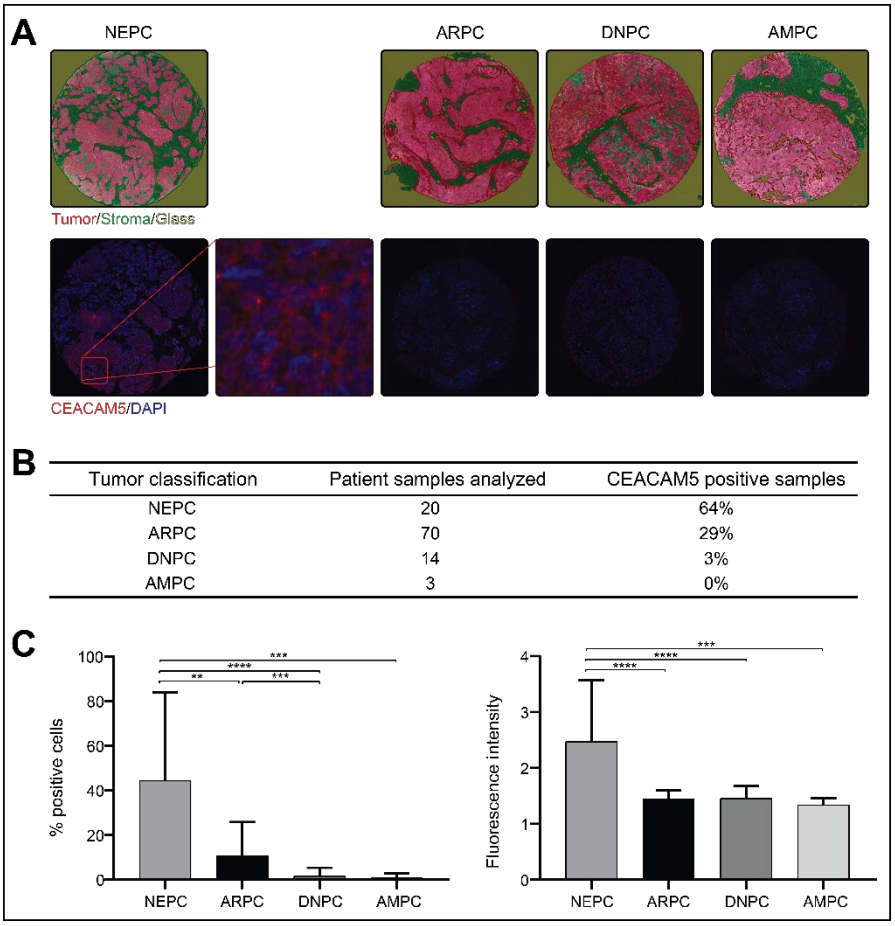
SA 2: Validate candidate cell surface markers on NEPC using proteomic approaches

Task 1: High-throughput cell surface proteomic analysis of prostate cancer cell lines (months 1-14) Completed during prior reporting periods.

Task 2: Validation of candidate cell surface proteins using low-throughput proteomic techniques (months 6-18) In progress. Multi-level validation of candidate NEPC cell surface marker expression by immunoblotting and immunohistochemistry of NEPC cell lines, xenografts, and archived tumors is nearly complete. Results related to carcinoembryonic antigen related cell adhesion molecule 5 (CEACAM5) were initially published in *Lee JK, et al. PNAS, 2018* in which CEACAM5 was found to be expressed in approximately 60% of NEPC tumors analyzed. We further expanded our analysis to understand the scope of CEACAM5 expression in prostate cancer by perform multiplex immunofluorescence analysis on a tissue microarray of lethal human metastatic castration-resistant prostate cancer (mCRPC) derived from samples obtained at rapid autopsy through the University of Washington (UW) Tissue Acquisition Necropsy (TAN) program. We specifically examined CEACAM5 expression in relation to other clinically relevant cell surface antigens like trophoblast cell surface antigen 2 (Trop2), prostate-specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA) across diverse pheno-

typic subtypes of end-stage mCRPC. We found that CEACAM5 expression in enriched in NEPC samples (Fig. 3A-C) and there is limited overlap between CEACAM5 and Trop2, PSMA, and PSCA expression at a single-cell level in NEPC (Fig. 4). These observations suggest that diagnostic and therapeutic agents aimed at Trop2, PSMA, and PSCA are unlikely to localize and effectively treat CEACAM5^{+} disease.

We previously reported that cooperative studies cooperative studies with Dr. Pete Nelson's laboratory at Fred Hutch also identified L1 cell adhesion molecule (L1CAM) as another promising cell surface antigen whose expression is enriched in NEPC. Further characterization of L1CAM expression in patient-derived xenograft models and archived clinical specimens is ongoing. We are also optimizing a multiplex immunofluorescence panel to perform analysis of the UW TAN tissue microarray to examine L1CAM expression relative to CEACAM5, delta-like 3 (DLL3), and NCAM1.



B

Tumor classification	Patient samples analyzed	CEACAM5 positive samples
NEPC	20	64%
ARPC	70	29%
DNPC	14	3%
AMPC	3	0%

Fig. 3. CEACAM5 is enriched in the NEPC subtype of mCRPC. (**A**) Representative immunofluorescence images of individual cores from the UW TAN TMA with tumor and stroma annotation as well as CEACAM5 and DAPI staining. Scale bar=200 µm. NEPC=neuroendocrine prostate cancer; ARPC=AR pathway-active prostate cancer; DNPC=double-negative (AR^{-}/NE^{-}) prostate cancer; AMPC=amphicrine (AR^{+}/NE^{+}) prostate cancer. (**B**) Tumor classification, number of samples (cores) per prostate cancer classification, and percent of CEACAM5 positive samples (defined as ≥10% with staining). (**C**) Percentage of cells with CEACAM5 staining and (**D**) fluorescence intensity of CEACAM5 staining based on tumor phenotype.

Task 3: *Prioritization of candidate NEPC cell surface proteins for therapeutic development (months 6-18)* Completed. We have prioritized candidate antigens based on restricted expression in normal tissues and expression in NEPC cell lines, patient-derived xenografts, and human samples. Our leading target cell surface antigens in NEPC are CEACAM5 and L1CAM at this juncture. Updates on therapeutic development related to these antigens are detailed below.

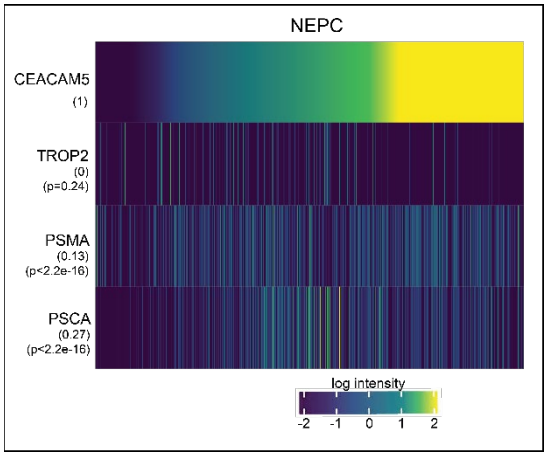


Fig. 4. Poor co-expression of Trop2, PSMA, or PSA with CEACAM5 in NEPC. Heatmap of log-transformed fluorescence intensities for staining of each of the cell surface antigens on a single-cell level in NEPC cores in the UW TAN TMA. The Pearson correlation coefficient and p-value are shown for each pairwise comparison with CEACAM5.

SA 3: *Develop novel antibody reagents targeting cell surface antigens in NEPC (months 18-48)*

Task 1: *Human scFv phage display library screening to identify antibody reagents (months 18-32)* In progress. CEACAM5 and L1CAM are glycosyl phosphatidyl inositol (GPI)-anchored membrane proteins for which therapeutic antibodies have previously been developed. We have used these antibodies as an accelerated starting point to evaluate the therapeutic targeting of these cell surface antigens in

NEPC. With respect to CEACAM5, we engaged in a sponsored research agreement with Immunomedics, Inc. to redirect their investigational anti-CEACAM5 antibody-drug conjugate labetuzumab govitecan to prostate cancer. Labetuzumab is a fully human monoclonal antibody with nanomolar affinity for CEACAM5 and labetuzumab govitecan has been investigated in a phase I/II study in patients with refractory/relapsed metastatic colorectal cancer [9]. Labetuzumab govitecan treatment was safe, tolerable, and associated with a median survival that exceeded matched, historic controls by approximately 4 months. For L1CAM, we obtained the murine CE7 antibody that binds the glycosylation-dependent CE7 epitope of L1CAM in neuroblastoma [10] from Dr. Michael Jensen's laboratory at Seattle Children's Research Institute. We have contracted with Creative Biolabs, Inc. to perform full humanization of the CE7 antibody by structural modeling and optimization of complementarity-determine region (CDR) grafting into a human antibody acceptor framework. The modeling has been completed and six candidate antibodies have been expressed and purified.

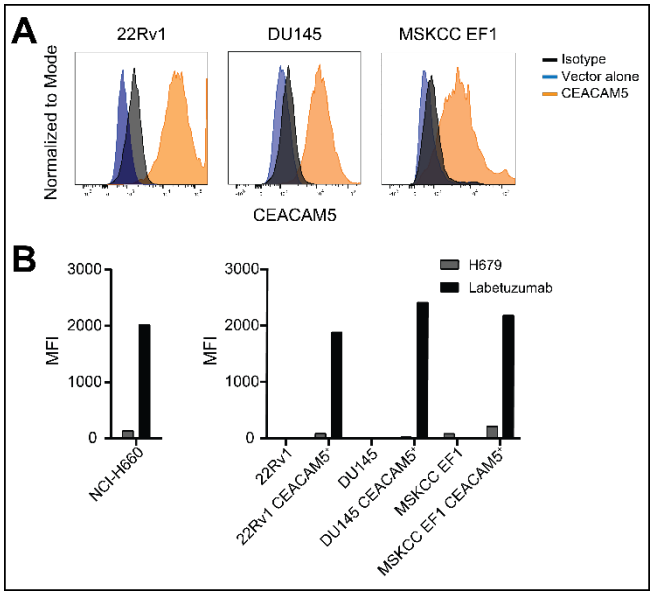


Fig. 5. Labetuzumab specifically binds CEACAM5 in prostate cancer cells. (**A**) Flow cytometry histogram plots demonstrating cell surface CEACAM5 expression in the 22Rv1, DU145, and MSKCC EF1 cell lines after stable transduction with CEACAM5-expressing lentivirus relative to cells transduced with control vector or isotype control antibody staining. (**B**) Mean fluorescence intensities of either labetuzumab or isotype control antibody H679 staining of the respective cell lines.

Task 2: *Validation of the specificity and performance of human monoclonal antibodies derived from phage display screening (months 18-34)* In progress. For CEACAM5, we tested the specificity of labetuzumab binding to isogenic pairs of CEACAM5$^-$ prostate cancer cell lines that were engineered by lentiviral transduction to express CEACAM5 (Fig. 5A-B) relative to an isotype control antibody. Labetuzumab but not the isotype control antibody H679 also bound to the natively CEACAM5$^+$ NCI-H660 NEPC cell line (Fig. 5B).

With respect to L1CAM, the six fully humanized antibody candidates are now undergoing validation for binding by flow cytometry on 1) an isogenic pair of IMR-32 neuroblastoma cell lines with native L1CAM expression or L1CAM knockout by CRISPR/Cas9 genome editing (Fig. 6A) and the 2) MSKCC EF1 NEPC cell line treated with DMSO or tunicamycin to inhibit N-linked glycosylation of the CE7 epitope (Fig. 6B). This approach may enable further screening of specificity not only for L1CAM expression but also for the glycosylated CE7 epitope.

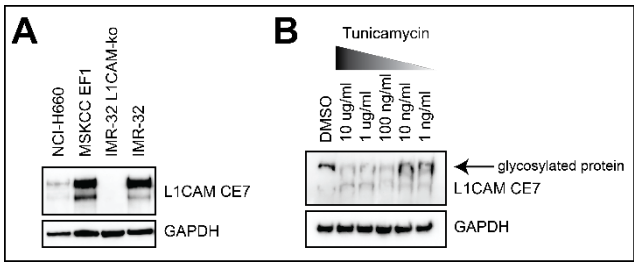


Fig. 6. Validation of cell line conditions to screen the specificity of fully humanized L1CAM CE7 candidate antibodies. L1CAM CE7 immunoblots for (**A**) NCI-H660, MSKCC EF1, IMR-32 with L1CAM knocked out (ko) by CRISPR/Cas9, or parental IMR-32 and (**B**) MSKCC EF1 cells treated with DMSO or a dose range of tunicamycin, an inhibitor of N-linked glycosylation.

Task 3: *Therapeutic evaluation of monoclonal antibodies against candidate cell surface targets (months 30-40)* In progress. We examined the genotoxicity of labetuzumab govitecan after incubation with isogenic pairs of CEACAM5$^-$ prostate cancer cell lines that were engineered by lentiviral transduction to stably express CEACAM5. Cells were incubated with labetuzumab govitecan on ice for 30 minutes, washed multiple times, and placed in growth media at 37ºC for 18 hours prior to collection and intracellular staining for γH2AX, a marker of double-stranded DNA breaks. We discovered that labetuzumab govitecan induces γH2AX as measured by flow cytometry in CEACAM5$^+$ prostate cancer cell lines in an antigen-dependent manner (Fig. 7A) that was generally consistent with the sensitivity of the cell lines to the SN-38 payload (Fig. 7B).

We also evaluated the therapeutic effects of a second-generation 4-1BB chimeric antigen receptor (CAR) T cell therapy harboring the L1CAM CE7 single-chain variable fragment (Fig. 8A) [11]

across a panel of prostate cancer cell lines. We identified specific T cell activation based on interferon-γ release upon co-culture of L1CAM CE7 CAR T cells with the MSKCC EF1 and NCI-H660 cell lines and the VCaP amphicrine (AR^+/NE^+) cell line (Fig. 8B). Furthermore, we demonstrated robust L1CAM CE7 CAR T cell killing of the $L1CAM^+$ MSKCC EF1 cell line while sparing the $L1CAM^-$ 22Rv1 and LNCaP cell lines (Fig. 8C).

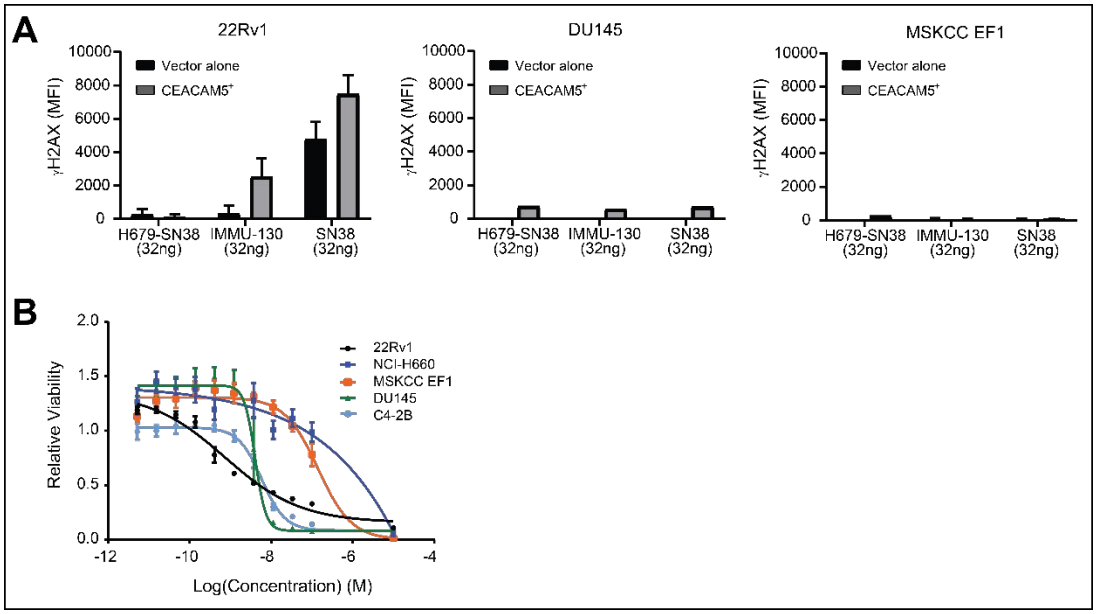


Fig. 7. Antigen-specific genotoxicity of labetuzumab govitecan (IMMU-130) in prostate cancer cell lines in a manner consistent with sensitivity to the payload SN-38. (**A**) Quantification of mean fluorescence intensity of γH2AX staining after 30 minutes of treatment of the respective cell lines with H679-SN38, IMMU-130, or SN38 on ice followed by an 18 hour incubation in growth media at 37ºC. (**B**) Dose-response curves of prostate cancer cell lines to SN-38.

Task 4: Mouse studies with therapeutic monoclonal antibodies in NEPC (months 40-48) <u>In progress.</u> We have completed studies investigating labetuzumab govitecan (IMMU-130) treatment of the $CEACAM5^+$ NCI-H660 NEPC cell line xenografts and LuCaP 49 and LuCaP 145.1 NEPC patient-derived xenografts in NSG mice. In the NCI-H660 studies, all mice treated with IMMU-130 25 mg/kg ip weekly (n=10) had complete tumor regression by day 17 while all mice treated with the equivalent dosing of the non-specific antibody-drug conjugate H679-SN38 (n=9) had undetectable tumors by day 24 (Fig. 9A). We next tested the LuCaP 49 and LuCaP 145.1 models with either IMMU-130 or H679-SN38 25 mg/kg ip every 4 days. In the LuCaP 49 studies, all mice treated with either IMMU-130 (n=10) or H679-SN38 (n=8) achieved complete

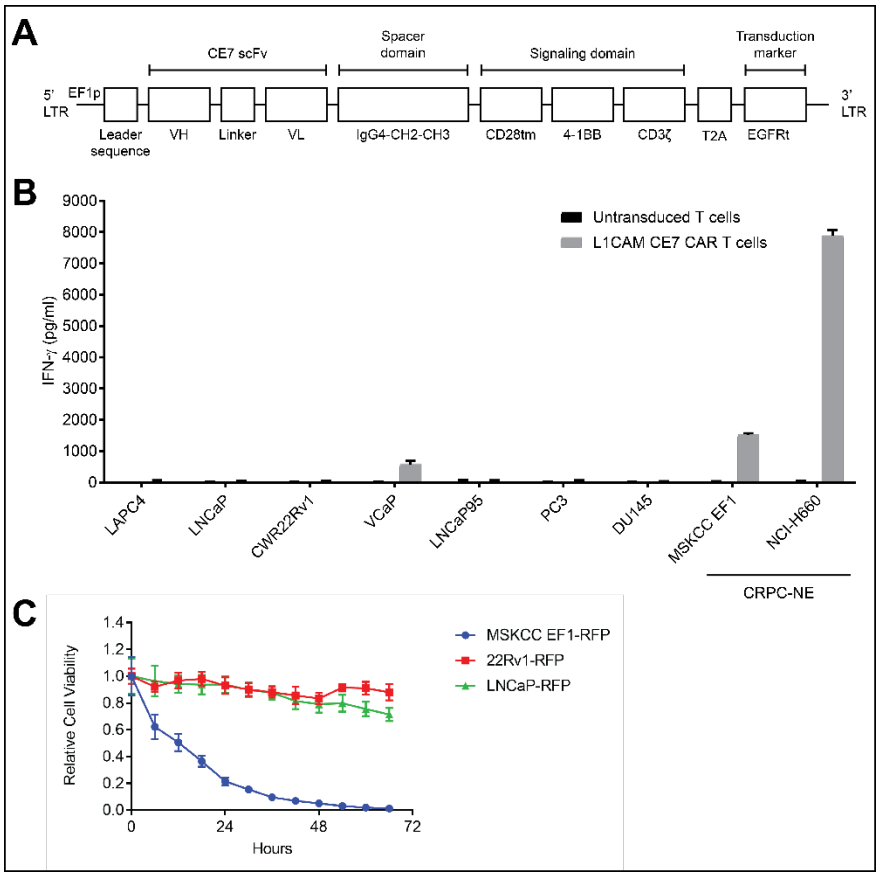


Fig. 8. Preliminary evidence of the antigen-specific activation of L1CAM CE7 CAR T cells and killing of NEPC cells. (**A**) Schematic of the second-generation 4-1BB L1CAM CE7 CAR construct. (**B**) Quantification of interferon-γ (IFN-γ) from co-cultures between either untransduced T cells or L1CAM CE7 CAR T cells with the respective prostate cancer cell lines at an effector-to-target (E:T) ratio of 1 after 24 hours. (**C**) Relative cell viabilities of MSKCC EF1, 22Rv1, or LNCaP cells co-cultured with L1CAM CE7 CAR T cells at an E:T ratio of 1 and enumerated with live cell fluorescence imaging.

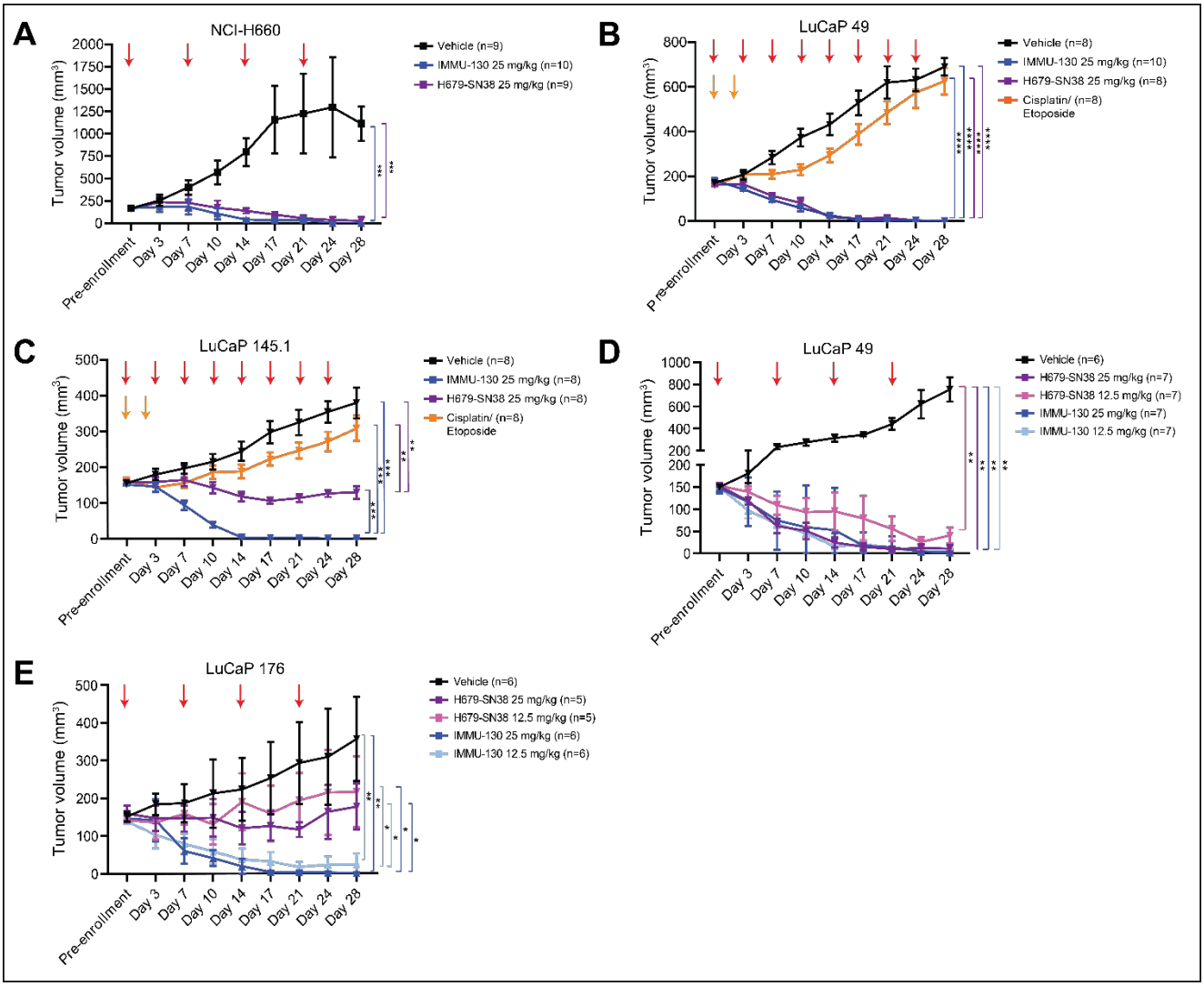


Fig. 9. Antitumor activity of labetuzumab govitecan (IMMU-130) in multiple preclinical models of CEACAM5⁺ NEPC. (**A-D**) Tumor volumes depicted over the 28 day treatment and observation period for prostate cancer models engrafted in NSG mice. Red arrows signify treatment days for vehicle, H679-SN38, and IMMU-130. Orange arrows signify treatment days for cisplatin/etoposide. Doses of H679-SN38 and IMMU-130 are identified in each respective legend.

tumor responses by day 14 (Fig. 9B). In contrast, mice bearing LuCaP 145.1 tumors demonstrated complete responses with IMMU-130 treatment (n=8) by day 14 while H679-SN38 treatment (n=8) inhibited tumor growth but did not eradicate any tumors (Fig. 9C).

Given the striking antitumor effects of IMMU-130, we examined the effects of treating CEACAM5$^+$ LuCaP 49 NEPC and LuCaP 176 AR^{low}/NE$^-$ patient-derived xenografts with a reduced dose (12.5 mg/kg) and administration schedule (weekly) of IMMU-130 or H679-SN38. In the LuCaP 49 studies, treatment with IMMU-130 at 25 mg/kg and 12.5 mg/kg resulted in tumor eradication in all mice (n=7) by day 21 (Fig. 9D). Both dose levels of H679-SN38 inhibited tumor growth but only the 25 mg/kg dose led to complete tumor responses. In the LuCaP 176 model, IMMU-130 treatment at 25 mg/kg led to tumor eradication in all mice (n=6) by day 17 while complete tumor responses were only observed in 50% of mice (n=6) treated at 12.5 mg/kg (Fig. 9E). Both doses of H679-SN38 slowed tumor growth but did not cause tumor regression.

Collectively, these results indicate that labetuzumab govitecan has significant antitumor activity in multiple clinically relevant patient-derived models of CEACAM5$^+$ NEPC. The effects of the control antibody-drug conjugate H679-SN38 in our studies are likely related to linker hydrolysis and systemic release of SN38. These findings indicate that labetuzumab govitecan has both regional antigen-specific and systemic non-specific antitumor effects. The benefits of such a moderately stable linker over an ultrastable linker may be increased efficacy in patients with tumor heterogeneity.

Key Research Accomplishments:

- Generated NEPC cell lines derived from the C4-2B and LNCaP cell lines by genetically defined NE lineage reprogramming. This NE transdifferentiation system has already provided significant functional insights into the roles of specific candidate factors in the NE lineage reprogramming of prostate cancer.

- Characterized CEACAM5 expression relative to other clinically relevant prostate cancer cell surface antigens.

- Demonstrated antitumor activity of labetuzumab govitecan across multiple cell line and patient-derived xenograft models of CEACAM5$^+$ NEPC. These findings have formed the basis for a planned multicenter phase I/II clinical trial of labetuzumab govitecan for men with CEACAM5$^+$ NEPC.

- Generated evidence for the specificity of the L1CAM CE7 single-chain variable fragment for NEPC based on antigen-specific CAR T cell activation and cytotoxicity.

- Completed modeling and expression of six fully humanized L1CAM CE7 antibody candidates which are undergoing screening for binding specificity.

Opportunities for Training and Professional Development:

This reporting period included my second year as an independent investigator in the Prostate Cancer Program in the Human Biology Division at Fred Hutch. My laboratory now includes three post-doctoral fellows, a graduate student, a technician, and a staff scientist that I oversee and mentor. I continue to meet with my junior faculty mentoring committee consisting of Drs. Pete Nelson, Valeri Vasioukhin, and Bob Eisenman.

My professional development now includes multiple areas of service to the institution including a member of the Cancer Consortium Scientific Review Committee, the Faculty Diversity and Inclusion Committee, and the Proteomics Faculty Advisory Committee. I am actively involved with the Prostate Cancer Foundation and the Pacific Northwest Prostate Cancer SPORE. I continue in my role as the co-leader of the Prostate Cancer Foundation Young Investigator Microenvironment and Immunology Working Group. In the past year, I also received the Eula and Donald S. Coffey Innovative Research Award from the Society for Basic Urologic Research and a Young Physician-Scientist Award from the American Society for Clinical Investigation. My new associations with these organizations have further expanded my professional network.

Importantly, I have developed a positive and productive relationship with preclinical/clinical collaborators at Immunomedics, Inc. which has helped move forward the evaluation of labetuzumab govitecan for the treatment of CEACAM5$^+$ NEPC.

Dissemination of Results:

The findings related to our recent work in targeting CEACAM5 in NEPC with labetuzumab govitecan will be shared at a seminar for the Pacific Northwest Prostate Cancer SPORE External Advisory Board meeting held on July 27, 2020 held at Fred Hutch. A manuscript summarizing these studies will be submitted in the next few weeks.

Plan for the Next Reporting Period:

In the next reporting period, we will continue studies related to the characterization of L1CAM expression in lethal mCRPC, screening and validation of fully humanized L1CAM CE7 antibodies, and mouse studies evaluating the efficacy of L1CAM CE7 CAR T cell therapy in mouse xenograft models of NEPC. If a fully humanized L1CAM CE7 antibody with specific binding is identified, we will also generate novel CARs based on the single-chain variable fragment and test the therapeutic efficacy of the antibody in cell lines studies.

IMPACT

The discovery of CEACAM5 expression in NEPC and the redirection and demonstration of therapeutic efficacy of labetuzumab govitecan in treating preclinical models of NEPC is now leading to clinical translation. We plan to initiate a multicenter phase I/II clinical trial of labetuzumab govitecan for men with CEACAM5^{+} NEPC with the approval and backing of Immunomedics, Inc. as the sponsor for this study. This is timely given the recent discontinuation of clinical development of the DLL3 antibody-drug conjugate rovalpituzumab tesirine for small cell lung cancer and NEPC due to toxicity. We have submitted a Prostate Cancer Foundation Challenge Award application to potentially fund correlative studies associated with this trial involving genomic, transcriptomic, and epigenetic characterization; the use of cell-free DNA as a biomarker; and determination of potential mechanisms of resistance to treatment.

Our finding of L1CAM expression in NEPC and the assembly of a multidisciplinary team with expertise in prostate cancer and targeting L1CAM in neuroblastoma using CAR T cell immunotherapy was recognized with a Movember Foundation-Prostate Cancer Foundation Challenge Award. We have generated preliminary data to support L1CAM as a target for L1CAM CE7 CAR T cell therapy in NEPC and are simultaneously beginning *in vivo* tumor challenge experiments and speaking with the Movember Foundation and Juno Therapeutics to determine whether they may collectively fund a phase I clinical trial.

CHANGES/PROBLEMS

The project was impacted by the COVID-19 pandemic and diminished workforce between the months of March and June 2020 due to physical distancing restrictions at the Fred Hutchinson Cancer Research Center. While the COVID-19 pandemic is still evolving and future closures may be unforeseen, I still believe that we are still on track to complete the proposed milestones.

One change to the project, as outlined previously, relates to pre-existing therapeutic antibodies for cell surface antigens that we validate in NEPC. While we initially proposed to generate new antibody reagents against these antigens, the use of existing or modifications of existing antibody reagents has already accelerated preclinical investigation and shortened the potential road to clinical translation.

PRODUCTS

None.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	*John K. Lee*
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Nearest person month worked:	*3*
Contribution to Project:	*Dr. Lee has performed data analysis and overseen the conduct of the study.*
Funding Support:	

Name:	*Tiffany Pariva*
Project Role:	*Research Technician*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Pariva has performed low-throughput proteomic analyses to validate the expression of candidate prostate cancer subtype-specific antigens in cell lines, xenografts, and archived clinical tissues. She also generated CAR T cells and performed co-culture experiments.*
Funding Support:	

SPECIAL REPORTING REQUIREMENTS

Nothing to Report.

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APPENDICES

None.