Regulation of CEACAM5 and Therapeutic Efficacy of an Anti-CEACAM5–SN38 Antibody–drug Conjugate in Neuroendocrine Prostate Cancer

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ABSTRACT

Purpose: Neuroendocrine prostate cancer (NEPC) is an aggressive form of castration-resistant prostate cancer (CRPC) for which effective therapies are lacking. We previously identified carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5) as a promising NEPC cell surface antigen. Here we investigated the scope of CEACAM5 expression in end-stage prostate cancer, the basis for CEACAM5 enrichment in NEPC, and the therapeutic potential of the CEACAM5 antibody–drug conjugate labetuzumab govitecan in prostate cancer.

Experimental Design: The expression of CEACAM5 and other clinically relevant antigens was characterized by multiplex immunofluorescence of a tissue microarray comprising metastatic tumors from 34 lethal metastatic CRPC (mCRPC) cases. A genetically defined neuroendocrine transdifferentiation assay of prostate cancer was developed to evaluate mechanisms of CEACAM5 regulation in NEPC. The specificity and efficacy of labetuzumab govitecan was determined in CEACAM5+ prostate cancer cell lines and patient-derived xenografts models.

Results: CEACAM5 expression was enriched in NEPC compared with other mCRPC subtypes and minimally overlapped with prostate-specific membrane antigen, prostate stem cell antigen, and trophoblast cell surface antigen 2 expression. We focused on a correlation between the expression of the pioneer transcription factor ASCL1 and CEACAM5 to determine that ASCL1 can drive neuroendocrine reprogramming of prostate cancer which is associated with increased chromatin accessibility of the CEACAM5 core promoter and CEACAM5 expression. Labetuzumab govitecan induced DNA damage in CEACAM5+ prostate cancer cell lines and marked antitumor responses in CEACAM5+ CRPC xenograft models including chemotheray-resistant NEPC.

Conclusions: Our findings provide insights into the scope and regulation of CEACAM5 expression in prostate cancer and strong support for clinical studies of labetuzumab govitecan for NEPC.

Introduction

While androgen deprivation therapy (ADT) is initially effective for the treatment of hormone-sensitive prostate adenocarcinoma, resistance is inevitable and leads to a state known as castration-resistant prostate cancer (CRPC). CRPC is heterogeneous and comprises multiple molecular phenotypes that diverge from conventional prostate adenocarcinoma and include neuroendocrine prostate cancer (NEPC) which is a high-grade, poorly differentiated, and lethal neuroendocrine carcinoma with unique patterns of DNA methylation, cancer (8), indicating that other factors may be involved. However, NEPC can vary in histologic appearance and neuroendocrine marker expression, likely due to molecular heterogeneity. Small-cell lung cancer (SCLC) shares many phenotypic characteristics with NEPC. Recently, four molecular subtypes of SCLC have been...
Here we characterize CEACAM5 expression in end-stage mCRPC relative to other cell surface antigens that are the active clinical focus of diagnostic and therapeutic development. We investigate the molecular basis for CEACAM5 expression in NEPC and uncover insights into the cancer differentiation-specific regulation of CEACAM5. Finally, we evaluate the antitumor activity of labetuzumab govitecan in preclinical models of CEACAM5+ CRPC, including NEPC, to justify the clinical investigation of this therapeutic agent in prostate cancer.

Materials and Methods

Cell lines

DU145 (catalog No. DU-145, RRID:CVCL_0105), 22Rv1 (catalog No. CRL-2505, RRID:CVCL_1045), C4-2B (catalog No. CRL-3315, RRID:CVCL_4784), and NCI-H660 (catalog No. CRL-5813, RRID:CVCL_1576) cell lines were purchased from the ATCC and LNCAp95 were a gift from Stephen R. Plymate (University of Washington, Seattle, WA). All cell lines were validated by short tandem repeat analysis after receipt. DU145, 22Rv1, C4-2B, and MSKCC FF1 (derived from the organoid line MSKCC-CaP4) were maintained in RPMI medium supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin, and 4 mmol/L GlutaMAX. NCI-H660 cells were maintained in Advanced DMEM/F12 medium supplemented with B27, 4 mmol/L GlutaMAX, and 10 ng/mL recombinant human basic FGF (bFGF) and EGF. Cell lines were cultured no more than 3 weeks after thawing prior to use in described experiments.

mIF of tissue microarrays

UW mCRPC TAN TMA (Prostate Cancer Biorepository Network) and FDA normal organ TMA (US Biomax Inc.) were used for mIF studies (Supplementary Tables S1–S3). Slides were stained on a Leica BOND Rx stainer (Leica) using Leica Bond reagents for antigen retrieval, antibody stripping (Epitope Retrieval Solution 2), and rinsing after each step (Bond Wash Solution). A high stringency wash was performed after the secondary and tertiary applications using high-salt TBST solution (0.05 mol/L Tris, 0.3 mol/L NaCl, and 0.1% Tween-20, pH 7.2–7.6). Opal Polymer HRP Mouse plus Rabbit (PerkinElmer) was used for all secondary applications.

H-scoring of CEACAM5 expression

H-scores were generated from the CEACAM5 mIF data using the CytoNuclear LC v2.0.6 module and HALO software. Briefly, individual cells were classified as having negative, weak, moderate, or strong CEACAM5 staining and assigned intensity scores of 0, 1, 2, and 3, respectively. The intensity score ranges were defined on the basis of CEACAM5 fluorescent intensity values as follows: 0 = 0 – positive CEACAM5 threshold value, 1 = threshold value – 25th quartile median, 2 = 25th quartile median – 75th quartile median, and 3 = 75th quartile median – maximum value reported. Intensity scores were then multiplied by the percentage of stained cells for a range of 0–300.

Serum CEA quantification

Cryopreserved serum samples obtained at rapid autopsy or a patient visit prior to rapid autopsy were obtained from the University of Washington Tissue Acquisition Necropsy (UW TAN) repository. CEA quantification was performed using a Clinical Laboratory Improvement Amendments–licensed CEA ELISA test (University of Washington Research Testing Services).
Regulation and Therapeutic Targeting of CEACAM5 in NEPC

Exome sequencing analysis
Paired-end exome sequencing [next-generation sequencing (NGS)] was performed using Illumina HiSeq or Illumina NovaSeq on genomic DNA isolated from rapid autopsy tissue samples. Sequence reads were aligned to the human reference genome hg19 using the Burrows-Wheeler Aligner (RRID:SCR_010990). GATK (RRID:SCR_001876) best practice was adopted to process all aligned BAM files. Germline and somatic mutation analyses were performed using ANNOVAR hg19 (RRID:SCR_012821) and manual curation was performed before determination of pathogenicity. Copy number was derived following the standardized Sequenza pipeline (RRID:SCR_016662). All copy-number calls were manually curated for potentially missed mid-sized structural aberrations (15–50 nt indels).

C4-2B neuroendocrine transdifferentiation assay
C4-2B cells were seeded in 6-well tissue culture plates at a density of 10^5 cells per mL in 3 mL of RPMI medium supplemented with 10% FBS, 100 U/mL penicillin and 100 µg/mL streptomycin, and 4 mM/L GlutaMAX. Cells were transduced approximately 4–6 hours after seeding at a defined multiplicity of infection of 4 for each lentivirus. Seventy-two hours after transduction, cells were trypsinized, washed, and transferred to 100-mm tissue culture plates in 15 mL of Advanced DMEM/F12 medium supplemented with B27, 4 mM/L GlutaMAX, and 10 ng/mL recombinant human bFGF and EGF. Media were replenished every 3–4 days. Cells were collected 11 days posttransduction for analysis.

Assay for transposase-accessible chromatin sequencing
Briefly, 50,000 cells were lysed in buffer containing NP-40, Tween-20, and digitonin. Nuclei were collected after centrifugation and transposed with Tn5 transposase for 30 minutes at 37°C. DNA was purified by MinElute Reaction Cleanup Kit (Qiagen) followed by PCR amplification to append indices/adapters, library purification, and quality control by Agilent TapeStation and library quantitation by qPCR. Assay for transposase-accessible chromatin sequencing (ATAC-seq) libraries underwent paired-end 50 bp sequencing on an Illumina NovaSeq 6000. Raw reads were processed with the ENCODE ATAC-seq pipeline (22) for quality control, alignment by Bowtie 2 (RRID:SCR_005476), and peak calling by MACS2 (RRID:SCR_013291). Inferred transcription factor activity was determined by HINT-ATAC (23) using HOCOMOCO (RRID:SCR_005409) and JASPAR (RRID:SCR_003030) binding motifs.

ATAC qPCR
ATAC-qPCR targeting the CEACAM5 core promoter peak was performed using ATAC libraries on the QuantStudio5 System (Thermo Fisher Scientific) with Applied Biosystems PowerUp SYBR Green Master Mix (Thermo Fisher Scientific). The mean cycle threshold (Ct) obtained for each promoter region was normalized to the AK5 control primers (24).

Immunoblot
Whole cell extracts were fractionated by SDS-PAGE and transferred to a nitrocellulose membrane using a transfer apparatus according to the manufacturer’s instructions (Invitrogen). Membranes were blocked with 5% nonfat milk in PBST (DPBS + 0.5% Tween 20) for 30 minutes while shaking, then incubated with primary antibodies at 4°C for 16 hours. Membranes were washed three times for 5 minutes with PBST and incubated with horseradish peroxidase (HRP)-conjugated anti-mouse or anti-rabbit secondary antibody for 1 hour at room temperature. Blots were washed three times for 5 minutes each with PBST and developed with Immobilon Western Chemiluminescent HRP Substrate (MilliporeSigma) for 3 minutes at room temperature. Blot images were acquired with a Chemidoc Imaging System (Bio-Rad) or autoradiography film.

CEACAM5 surface protein detection by flow cytometry
DU145, 22Rv1, and MSKCC EF1 cells were dissociated with Versene-EDTA (Thermo Fisher Scientific) into single-cell suspensions. Cells were washed once with mAb wash buffer (MW; PBS + 0.1% FBS + 0.1% sodium azide) then resuspended in 100 µL MW and 5 µL of anti-CEACAM5-APC or IgG isotype-APC per 10^6 cells and incubated at room temperature in the dark for 30 minutes. Cells were washed once with MW, resuspended in MW, acquired on a BD FACSCanto II (BD Biosciences), and analyzed with FlowJo (v10; RRID:SCR_008520).

Labetzumab cell surface binding
DU145, 22Rv1, and MSKCC EF1 cell lines expressing empty vector or CEACAM5 vector were dissociated nonenzymatically with Versene-EDTA into single-cell suspensions. Cells were washed once with PBS and resuspended in 100 µL of 1 µg/mL of h679 or labetzumab (Immunomedics, Inc.) and incubated at 4°C on ice for 1 hour. Cells were then washed twice with PBS, incubated with an anti-human IgG-PE-Cy5 secondary antibody (Thermo Fisher Scientific) at 4°C on ice for 30 minutes, washed with PBS, acquired on a SH800 (Sony), and analyzed with FlowJo (v10).

βH2AX detection of dsDNA breaks
DU145, 22Rv1, and MSKCC EF1 cells were dissociated nonenzymatically with Versene-EDTA (Thermo Fisher Scientific), washed with PBS, resuspended in PBS and prechilled on ice at 4°C for 20 minutes, followed by incubation with labetzumab govitecan or h679-SN-38 (Immunomedics, Inc.), or SN-38 (Sigma) for 30 minutes on ice at 4°C. Cells were then washed six times with cold PBS, and cultured for 16 hours in culture media at 37°C. For extended SN-38–treated conditions, cells were cultured at 37°C in media containing SN-38 for 16 hours. Cells were then dissociated with trypsin 0.25%, washed with MW, fixed with BD Cytofixation Buffer (BD Biosciences), permeabilized with BD PhosflowPerm Bufffer II (BD Biosciences), and stained with anti-βH2AX-BV421 or IgG isotype control, as per manufacturer’s instructions. Cells were washed twice with MW, resuspended in MW, acquired on a BD FACSCanto II (BD Biosciences), and analyzed with FlowJo (v10).

SN-38 dose responses in prostate cancer cell lines
DU145, 22Rv1, MSKCC EF1, and NCI-H660 cells were seeded at 5 × 10^5 cells (50 µL) per well in 96-well flat bottom, tissue culture–treated, white plates (Corning). Cells were treated with serial dilutions of SN-38 (50 µL) in replicates of 8, diluted in appropriate culture media, at 37°C for 96 hours. Cell viability was determined using the CellTiter-Glo 2.0 Assay (Promega).

IHC of LuCap patient-derived xenograft tumors
Formalin-fixed, paraffin-embedded tissue sections were baked at 65°C for 1–2 hours, deparaffinized in xylene, and rehydrated in 100%, 95%, and 70% ethanol. Tissue sections were heated in antigen retrieval buffer (0.2 mol/L citric acid and 0.2 mol/L sodium citrate) within a pressure cooker followed by PBS wash. Tissue slices were blocked with 2.5% horse serum for 30 minutes and then incubated with primary antibody diluted in 2.5% horse serum overnight at 4°C. HRP was
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detected with ImmPRESS-HRP anti-mouse or anti-rabbit IgG peroxidase detection kits (Vector Laboratories) and staining was visualized with DAB peroxidase substrate (Dako). Tissue sections were counterstained with hematoxylin and dehydrated for mounting.

**Mouse xenograft studies**

All animal care and studies were performed in accordance with an approved Fred Hutchinson Cancer Research Center Institutional Animal Care and Use Committee protocol and Comparative Medicine regulations. Six-week-old, male NSG (NOD-SCID-IL2Rγ-null, RRID: BCBC_4142) mice were obtained from the Jackson Laboratory. A total of 5 × 10⁶ cells from each prostate cancer cell line were suspended in 100 μL of cold Matrigel (Corning) and implanted by injection subcutaneously into NSG mice. For LuCaP patient-derived xenografts (PDX), a 1-mm³ piece of prostate tumor tissue was surgically implanted subcutaneously into NSG mice. Mice were enrolled into a treatment arm when tumors reached 150 mm³ and treated by intraperitoneal injection at the frequency and with the doses described. Labetuzumab govetican and b679-SN-38 doses were prepared fresh through reconstitution with 0.9% preservative-free sodium chloride (McKesson Medical-Surgical). Cisplatin and etoposide (NH Developmental Therapeutics Program, RRID:SCR_003057) were prepared and stored at room temperature and 4 °C, respectively. Mice were monitored biweekly for tumor growth, weight, and body condition score. A complete response is defined as an undetectable tumor.

**Complete blood counts and serum chemistries**

Retro-orbital bleeds yielding approximately 200 μL of blood were performed on mice prior to receiving the first dose at enrollment on day 0, as well as on days 14 and 28 of the study. Blood was collected into green top lithium heparin microcontainers (Becton Dickinson) and stored at 4°C for up to 24 hours (Phoenix Labs).

**Statistical methods**

All data are shown as mean ± SD. For sample sizes less than 40, normality testing was performed with the D’Agostino-Pearson test. For single comparisons, statistical analyses were performed using a two-sided Student t test. For multiple comparisons, statistical analyses were performed using ANOVA with Tukey post hoc correction. Data not normally distributed were alternatively analyzed using a two-sided Kruskal–Wallis nonparametric test or Brown–Forsythe and Welch ANOVA with Games-Howell nonparametric post hoc correction. For correlation analysis, Pearson correlations or Spearman rank correlations were performed for normal and not normal data, respectively. Best-fit curves were generated with linear regression modeling. Significance was defined as P ≤ 0.05.

All studies were conducted in accordance with the ethical guidelines expressed in the World Medical Association Declaration of Helsinki.

**Data and material availability**

Raw and analyzed RNA sequencing (RNA-seq) and ATAC-seq data are available at GEO accession number GSE154576. All other materials will be available upon request and completion of a Material Transfer Agreement.

**Results**

**Enrichment of CEACAM5 protein expression in NEPC**

To examine CEACAM5 expression across phenotypic subtypes of advanced prostate cancer, we performed immunofluorescence (IF) staining on a clinically and histologically annotated tissue microarray (TMA) of lethal mCRPC tissues from 34 patients collected at rapid autopsy through the UW TAN program (25). Two of 34 patient samples were excluded because of poor-quality cores, allowing for the complete analysis of 32 patient tissues. Tissues were classified into four tumor subtypes based on IHC staining for AR, PSA, chromogranin A (ChrA), and synaptophysin (SYP): (i) AR-positive prostate cancer (ARPC: AR⁺ or PSA⁺, ChrA⁺, and SYP⁺); (ii) NEPC (AR⁺ and PSA⁺, ChrA⁺ or SYP⁺); (iii) double-negative prostate cancer (DNPC: AR⁺, PSA⁺, ChrA⁺, and SYP⁺); or (iv) amphiicrine prostate cancer (AMPC: AR⁺ or PSA⁺ and ChrA⁺ or SYP⁺). Stromal regions of tissue cores were classified on the basis of morphology (Fig. 1A) and excluded from all analyses to focus on tumor parenchyma. Image analysis revealed that the overall level of CEACAM5 expression was heightened in NEPC based on fluorescence intensity (Fig. 1B) and that NEPC cores contained significantly more CEACAM5⁺ cells (44% ± 39.6%; Fig. 1C). Integrated CEACAM5 H-scores (% cells stained × staining intensity) were substantially higher in NEPC (81 ± 87.5; Fig. 1D) compared with other prostate cancer subtypes. CEACAM5 expressed on the surface of cells is often shed into the bloodstream and can be measured as serum CEA. Serum CEA is a common clinical cancer biomarker but has had a relatively limited role in the clinical management of prostate cancer. Elevation of serum CEA combined with neuroendocrine tumor marker expression has previously been reported as a clinical criterion for aggressive variant prostate cancer, a spectrum of prostate cancers including NEPC that are molecularly characterized by combined defects in TP53, RB1, and PTEN and respond poorly to AR-directed therapies (26). To explore the relationship between serum CEA levels and tumor CEACAM5 expression in lethal mCRPC subtypes, we assayed banked serum samples collected concurrently with tumor tissue from 18 of the 34 patients represented in the UW mCRPC TAN TMA. We found a significant correlation between serum CEA levels and tumor CEACAM5 expression (r = 0.40) based on H-score (Fig. 1E). The correlation appeared to be driven primarily by patients with NEPC compared with other mCRPC subsets (Supplementary Fig. S1A and S1B) but subgroup analysis was not statistically significant potentially due to limited sample size. These data suggest that serum CEA could be a valuable adjunct clinical biomarker of NEPC and should be investigated further as a part of prospective clinical trials.

Genomic profiling of prostate cancer by NGS has identified distinct molecular disease subtypes (27). We performed a limited exploratory analysis of whole-exome sequencing of 38 prostate cancer tissues (17 CEACAM5⁺ and 21 CEACAM5⁻) from 28 of 34 patients represented on the UW mCRPC TAN TMA. Our analysis focused on a subset of genes commonly altered in mCRPC including RB1 and TP53 and genes in the PI3K/AKT signaling pathway (Supplementary Table S4). Monoallelic or biallelic copy loss of RB1, TP53, and PTEN appeared to be equally common in CEACAM5⁺ and CEACAM5⁻ mCRPC tissues, at frequencies consistent with prior reports (8, 28). Predicted functional mutations were observed in RB1 and TP53, and the mutational frequency was similar in CEACAM5⁺ and CEACAM5⁻ tissues. Monoallelic or biallelic copy loss of FOXO3, MAP3K7, and RRAGD was enriched in CEACAM5⁺ samples compared with CEACAM5⁻ samples by a factor of 2. MAP3K7 loss has specifically been reported to promote the development of clinically aggressive prostate cancer, and is associated with AR loss and neuroendocrine differentiation (29).

As tissues were collected from multiple metastatic sites (Supplementary Tables S1 and S2) and variable CEACAM5 expression was identified within tissues, we next characterized the intratumor phenotypic heterogeneity of mCRPC in the NEPC samples from the UW...
mCRPC TAN cohort. Four of eight (50%) patients with NEPC had mixed disease based on the presence of additional histologic phenotypes at other tumor sites (Fig. 1F). To evaluate CEACAM5 expression in the context of this intrapatient heterogeneity, we examined all cores from each of these eight NEPC patients. Five of eight patients (62.5%) were found to have CEACAM5+ NEPC (patients 2, 5, 6, 7, and 8). In these five cases, CEACAM5 expression was present at all NEPC tissue sites, albeit with variability in the frequency of CEACAM5+ cells between sites (Fig. 1F). In addition, the metastatic samples within these five patients that lacked CEACAM5 expression exhibited non-NEPC phenotypes (Fig. 1F). These data further demonstrate enhanced CEACAM5 expression in NEPC, not only across a diverse series of patients, but also within patients harboring phenotypically heterogeneous mCRPC.

We also profiled CEACAM5 expression by IF in a normal human organ TMA (Supplementary Tables S1 and S3). Consistent with prior
CEACAM5 expression was detectable at low levels in multiple healthy tissues including the lung, stomach, small intestine, and colon (Supplementary Fig. S2A–S2C; refs. 14, 30, 31). However, the intensity of CEACAM5 staining in normal organs was significantly lower than in NEPC samples represented in the UW mCRPC TAN TMA (Fig. 1D). This difference in expression could signify a therapeutic window for agents directed at CEACAM5 when applied to NEPC. Collectively, these results provide a comprehensive assessment of

Figure 2.
CEACAM5 expression relative to other targetable prostate cancer cell surface antigens. Percentage of cells expressing CEACAM5, Trop2, PSMA, and PSCA (A) and staining intensity from mIF of ARPC (n = 70), NEPC (n = 20), DNPC (n = 14), and AMPC (n = 3) tissue cores (B). C, Coexpression of PSMA and PSCA in Trop2+ cells per core. D, Co-expression of Trop2, PSMA, and PSCA in CEACAM5+ cells per core. E, Quantitative single-cell mIF signal intensities of proteins (rows) in cells from ARPC (n = 655,676) and NEPC cores (n = 113,509, F). Error bars represent ± SD. *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001. Kruskal–Wallis P values are shown for plots A–D. Pearson correlation coefficient (r) and P values for each measured protein is shown numerically next to heatmap rows.
CEACAM5 expression in patients with lethal mCRPC, including NEPC, and in healthy human tissues.

**CEACAM5 expression relative to other targetable cell surface antigens in prostate cancer**

Multiple clinically relevant prostate cancer antigens including trophoblast cell surface antigen 2 (Trop2), prostate-specific membrane antigen (PSMA), and prostate stem cell antigen (PSCA) are the focus of intense clinical development for mCRPC. The Trop2-directed ADC sacituzumab govitecan (IMMU-132) is currently being evaluated in a phase II study for mCRPC (32). PSMA bispecific T-cell engagers, PSMA radioligand therapies, and PSMA PSCA chimeric antigen receptor T-cell therapies are also under clinical investigation for mCRPC. We focused on characterizing the coexpression of CEACAM5 and these prostate cancer antigens in lethal mCRPC using a multiplex IF (mIF) staining panel on the UW mCRPC TAN TMA (Supplementary Fig. S3). mIF image analysis demonstrated inverse patterns of (i) CEACAM5 and (ii) Trop2, PSMA, and PSCA staining frequencies and intensities in NEPC and ARPC tissue cores (Fig. 2A and B). Specifically, CEACAM5 expression was enriched in NEPC while Trop2, PSMA, and PSCA expression was heightened in ARPC. Furthermore, PSMA and PSCA were frequently expressed in Trop2 positive cores in ARPC but not in NEPC, DNPC, or AMPC (Fig. 2C). These results are consistent with the prior characterization of Trop2 as an epithelial marker and the established androgen-regulated nature of PSMA and PSCA expression (33, 34). In contrast, Trop2, PSMA, and PSCA were much less frequently expressed in CEACAM5+ cores in NEPC (Fig. 2D).

We evaluated mIF at a single-cell level across all ARPC and NEPC tissue cores to investigate more granular, digital relationships between (i) Trop2, PSMA, and PSCA coexpression in ARPC and (ii) CEACAM5, Trop2, PSMA, and PSCA coexpression in NEPC. Trop2 and PSMA (r = 0.42) but not PSCA (r = 0.01) expression were correlated in ARPC cells (Fig. 2E). On the other hand, CEACAM5 did not correlate with Trop2 (r = 0) or PSMA (r = 0.13) and weakly correlated with PSCA (r = 0.27) expression in NEPC cells (Fig. 2F).

The variable coexpression of Trop2, PSMA, and/or PSCA indicate the presence of highly heterogeneous ARPC cell populations in lethal mCRPC. Furthermore, these findings suggest that diagnostic and therapeutic modalities under investigation to target Trop2, PSMA, and PSCA in prostate cancer may not effectively localize and treat CEACAM5+ NEPC.

**Association between ASCL1 and CEACAM5 expression in NEPC**

CEACAM5 is highly expressed in colorectal cancer where prior studies have implicated TGFβ and retinoic acid signaling in CEACAM5 transcriptional regulation (35, 36). However, little is known about the regulation of CEACAM5 expression in other cancer types including NEPC. On the basis of published literature, we discovered that CEACAM5 is expressed in some neuroendocrine carcinomas such as medullary thyroid carcinoma (MTC) and SCLC but not others like Merkel cell carcinoma (37, 38). MTC arises from parafollicular cells which represent calcitonin-secreting neuroendocrine cells of the thyroid that require ASCL1 for their development (39). In SCLC, CEACAM5 expression is specifically enriched in the ASCL1high subtype over other subtypes including NeuroD1high SCLC (Supplementary Fig. S4A and S4B). In contrast, Merkel cell carcinoma does not express ASCL1 and instead uniformly expresses NeuroD1 (37, 40).

On the basis of these associations in other neuroendocrine carcinomas, we postulated that ASCL1 may regulate CEACAM5 expression in NEPC. To explore this possibility, we first examined the two available cell line models of NEPC, NCI-H660, and MSKCC EFl. Previously, we have shown that NCI-H660 cells express CEACAM5 and MSKCC EFl cells do not (14). Transcriptome profiling revealed differential enrichment of ASCL1 in NCI-H660 and NEUROD1 in MSKCC EFl cells (Fig. 3A), consistent with our hypothesis. We further examined gene expression data from Stand Up To Cancer (SU2C) mCRPC biopsies (41), UW mCRPC TAN rapid autopsies (42), and the LuCap PDX series (42) to scrutinize CEACAM5, ASCL1, and NEUROD1 expression in NEPC. Across these three datasets, CEACAM5 expression generally associated with ASCL1 expression but not NEUROD1 expression in NEPC samples (Fig. 3B). In the SU2C dataset, CEACAM5 expression was strongly correlated with ASCL1 (r = 0.95), but not NEUROD1 (r = 0.12) across mCRPC samples demonstrating a neuroendocrine score of >0.4 consistent with NEPC (Fig. 3C and E). The Beltran 2016 NEPC cohort (9) also showed a positive correlation for CEACAM5 and ASCL1 (r = 0.75) and interestingly NEUROD1 to a lesser extent (r = 0.44; Fig. 3D and F). The correlation between ASCL1 and NEUROD1 expression was negative (r = −0.27) in the SU2C dataset while the same comparison showed a positive correlation (r = 0.39) in the Beltran dataset (Fig. 3G and H). These findings may reflect increased representation of mixed ASCL1high and NeuroD1high NEPC tumors in the Beltran dataset. Of note, Delta-like 3 (DLL3) is a Notch ligand enriched in NEPC (43) that is the target of multiple therapeutics in clinical development for SCLC and is known to be regulated by ASCL1 (44). CEACAM5 expression correlated with DLL3 expression in the SU2C (r = 0.54) and Beltran 2016 NEPC (r = 0.46) datasets (Supplementary Fig. S5A and S5B), suggesting that both genes might be regulated by similar programs.

**Regulation of CEACAM5 expression during neuroendocrine transdifferentiation of prostate cancer**

To uncover possible cis-regulatory elements involved in the transcriptional regulation of CEACAM5 in prostate cancer, we examined chromatin accessibility of the CEACAM5 gene locus using ATAC-seq in multiple prostate cancer cell lines, including the NEPC cell lines NCI-H660 and MSKCC EFl and the AR+ cell lines 22Rv1 and LNCaP95. We identified a differential chromatin accessibility peak located at −191 to −92 upstream of the CEACAM5 transcriptional start site encompassing FANTOM5 Cap Analysis of Gene Expression tags of promoter elements in the CEACAM5+ NCI-H660 cell line but not in the CEACAM5− MSKCC EFl, 22Rv1, or LNCaP95 cell lines (Fig. 4A). This peak overlaps with the previously described core promoter region spanning −403 to −124 of the CEACAM5 gene locus (45) and was also prominent in pan-cancer The Cancer Genome Atlas ATAC-seq data (46) in tumor types where CEACAM5 is expressed including colorectal (COAD), esophageal (ESCA), gastric (STAD), and breast cancer (BRCA; Fig. 4A). Consistent with these findings, a coinciding DNase I hypersensitivity site was observed in CEACAM5+ normal colon tissues but not in CEACAM5− normal breast tissues analyzed by the Encyclopedia of DNA Elements (ENCODE) Project (Supplementary Fig. S6). In addition, the peak heights of the DNase I hypersensitivity site corresponded to reported levels of CEACAM5 expression in colorectal and breast cancer cell lines (Supplementary Fig. S6).

Inferred transcription factor binding from ATAC-seq indicated enhanced activity of ASCL1 in NCI-H660 cells and NeuroD1 in MSKCC EFl cells (Fig. 4B) which is in concert with their differential expression in these cell lines. However, functional validation studies with short hairpin RNA (shRNA)-mediated knockdown of ASCL1 in NCI-H660 cells and ectopic expression of ASCL1 in MSKCC EFl cells
Figure 3.
Association of ASCL1 and CEACAM5 expression in NEPC. A, RNA-seq gene expression heatmap of ASCL1 and NEUROD1 in NCI-H660, MSKCC EF1, 22Rv1, and LNCaP95 cell lines. B, RNA-seq gene expression heatmap of NEPC samples (columns) from the Stand Up To Cancer (SU2C) mCRPC cohort, the University of Washington Tissue Acquisition Necropsy (UW TAN) lethal mCRPC cohort, and LuCaP patient-derived xenograft lines. C and D, Correlation dot plots of CEACAM5 and ASCL1, CEACAM5 and NEUROD1 (E-F), and ASCL1 and NEUROD1 (G-H) gene expression in NEPC samples defined by a neuroendocrine gene signature score >0.4 in the SU2C dataset (n = 10) and the Beltran 2016 NEPC dataset (n = 15). Pearson correlation coefficients (r) are shown for correlative gene expression analyses.
Figure 4.
Regulation of CEACAM5 expression during neuroendocrine transdifferentiation. A, Integrative Genomics Viewer tracks showing an ATAC-seq peak at the promoter (orange arrow) upstream of the transcriptional start site of CEACAM5. B, Lineplots demonstrating inferred ASCL1 and NeuroD1 activity in the NCI-H660 and MSKCC EF1 cell lines using differential transcription factor binding motif footprinting of ATAC-seq data. C, Immunoblots demonstrating CEACAM5 protein expression in NCI-H660 cells with ASCL1 knockdown by shRNA and in MSKCC EF1 cells with ectopic ASCL1 expression. D, Immunoblots showing CEACAM5 and neuroendocrine differentiation marker expression in C4-2B cells overexpressing ASCL1, NeuroD1 (E), or ASCL2 (F) in the context of p53 R175H, Rb1 knockdown, and/or overexpression of N-Myc. Chromatin accessibility of the CEACAM5 promotor determined by ATAC-qPCR in NCI-H660 cells relative to MSKCC EF1 cells (G) and C4-2B control cells and cells reprogrammed with ASCL1, ASCL2, or NeuroD1 (H). P = p53 R175H; R = shRB1; N = N-Myc; A = ASCL1. Histograms depict means ± SD for biological replicates each with two technical replicates. *, P < 0.05. Student t test P values are shown for G and Kruskal–Wallis P values are shown for H.
had no discernable effect on CEACAM5 expression (Fig. 4C). ASCL1 and NeuroD1 knockdown in the respective NCI-H660 and MSKCC EF1 cell lines was detrimental to cell viability compared with controls (Supplementary Fig. S7A–S7C), indicating perhaps that these lines are genetically hardwired and intolerant of perturbations to these transcription factors. The data could also imply that ASCL1 may not regulate CEACAM5 expression through direct transactivation. To corroborate this idea, we examined published ASCL1 chromatin immunoprecipitation followed by sequencing (ChIP-seq) data across multiple studies from ASCL1<sup>high</sup> SCLC cell lines (12, 47, 48), including the NCI-H889 and NCI-H1755 cell lines which express outlier levels of CEACAM5 (Supplementary Fig. S8A). These analyses indicate the absence of ASCL1 binding peaks near the CEACAM5 gene locus (Supplementary Fig. S8B) but the presence of previously characterized

Figure 5.
Labetuzumab govitecan induces dsDNA damage in a CEACAM5-specific manner. A, CEACAM5 surface protein expression determined by flow cytometry in prostate cancer cell lines transduced with lentiviral expression constructs. B, Labetuzumab binding to CEACAM5 in prostate cancer cell lines. Measurement of intracellular γH2AX staining of 22Rv1 (C), DU145 (D), and MSKCC EF1 (E) cells 16 hours after treatment with h679-SN-38, labetuzumab govitecan, or SN-38 for 30 minutes on ice. MFI, mean fluorescence intensity. Histograms depict means ± SD for experimental duplicates.
peaks associated with genes bound by ASCL1 such as DLL3 and BCL2 (refs. 12, 44; Supplementary Fig. S8C and S8D). We therefore hypothesized that ASCL1, as a pioneer neural transcription factor, may epigenetically regulate CEACAM5 by chromatin remodeling. We also reasoned that genetic studies in the hardwired NCI-H660 and MSKCC EF1 NEPC cell lines may not recapitulate dynamic epigenetic regulation of CEACAM5 expression that occurs during the progression of human prostate cancer.

As an alternative approach, we developed a genetically defined system to induce neuroendocrine transdifferentiation of prostate cancer. We introduced ASCL1 and other factors causally associated with neuroendocrine transdifferentiation of prostate cancer including dominant-negative TP53 R175H, shRNA targeting RBP1 (shRBP1), and MYCN either alone or in combination into the androgen-independent ARPC cell line C4-2B. While C4-2B cells do not express CEACAM5 at baseline, we discovered that all conditions in which ASCL1 was introduced stimulated expression of CEACAM5 and the neuroendocrine markers SYP and insulinoma-associated protein 1 (INSM1; Fig. 4D). In contrast, all other C4-2B conditions in which ASCL1 was omitted did not exhibit neuroendocrine differentiation (Fig. 4D). We discovered that ectopic expression of NeuroD1 within this system also induced CEACAM5, SYP, and INSM1 expression (Fig. 4E). Notably, expression of ASCL1 and/or NeuroD1 downregulated AR and AR-dependent NK3 homeobox 1 (NKX3-1) expression (Fig. 4E), indicating that these factors may be critical in orchestrating lineage reprogramming from ARPC to NEPC. We also observed overexpression of NeuroD1-induced ASCL1 expression and the introduction of both ASCL1 and NeuroD1 further enhanced CEACAM5 expression (Fig. 4E).

We evaluated a second ASCL1 family member, ASCL2, in the C4-2B cell line to determine whether these effects may be specific to ASCL1. ASCL2 is also a pioneer transcription factor involved in the specification of multiple lineages including trophoblast (49), Th cells (50), and intestinal stem cells (51). Furthermore, ASCL2 expression is associated with the nonneuroendocrine cell line 22Rv1, the AR þ line DU145, and the NEPC line MSKCC EF1 (Fig. 5A). These data confirm the specificity of labetuzumab binding and the genotoxicity of labetuzumab in prostate cancer cell lines which generally correlates with the relative sensitivities of the lines to SN-38.

In vitro specificity and cytotoxicity of labetuzumab govitecan in NEPC

We previously reported that a CEACAM5 chimeric antigen receptor T-cell therapy demonstrates antitumor activity in NEPC cell line models (14). However, we recognized the lengthy time horizon and numerous hurdles to advancing this type of cancer treatment to the clinic. We therefore concentrated on studies to target CEACAM5 in prostate cancer by redirecting the established CEACAM5 ADC labetuzumab govitecan with the anticipation that compelling results could lead to an accelerated path to clinical translation. We first characterized the specific binding of labetuzumab, the humanized antibody component of labetuzumab govitecan, to prostate cancer cell lines with native and engineered expression of CEACAM5. CEACAM5 was stably expressed in three CEACAM5 þ prostate cancer cell lines: the AR þ line 22Rv1, the AR þ line DU145, and the NEPC line MSKCC EF1 (Fig. 5A). We detected labetuzumab binding in all four cell lines expressing CEACAM5 as well as the natively CEACAM5 þ NCI-H660 cell line, but not in isogenic negative control cell lines (Fig. 5B).

We then investigated the genotoxic effects of labetuzumab govitecan on the prostate cancer cell line panel by measuring γH2AX, a marker of double-stranded DNA (dsDNA) breaks. Cells were incubated with labetuzumab govitecan for 30 minutes, extensively washed to remove unbound drug, and propagated in cell culture for 16 hours prior to staining and analysis. Labetuzumab govitecan provoked greater γH2AX signal in the CEACAM5 þ 22Rv1 cell line relative to the control 22Rv1 cell line and compared with incubation with the nonspecific ADC, h679-SN-38 (Fig. 5C). In contrast, SN-38 alone induced γH2AX in an antigen-independent manner in both the CEACAM5 þ and CEACAM5 À 22Rv1 cell lines (Fig. 5C). h679-SN-38, labetuzumab govitecan, and SN-38 did not generate substantial γH2AX signal in the DU145 and MSKCC EF1 cell lines, irrespective of CEACAM5 expression status (Fig. 5D and E). To determine the overall susceptibility of the cell lines to SN-38, we assessed γH2AX levels following a longer exposure to SN-38 in culture. After a 16-hour incubation, SN-38 induced γH2AX in all three cell lines (Supplementary Fig. S10A–S10C), albeit to different extents consistent with drug sensitivity based on IC50 calculations from dose–response curves in each of the cell lines with the exception of DU145 (Supplementary Fig. S10D). These data confirm the specificity of labetuzumab binding and the genotoxicity of labetuzumab govitecan in CEACAM5 þ prostate cancer cell lines which generally correlates with the relative sensitivities of the lines to SN-38.

In vivo antitumor activity of labetuzumab govitecan in NEPC

We first examined the antitumor activity of labetuzumab govitecan in vivo using CEACAM5 þ NCI-H660 NEPC cell line xenograft tumors established in NOD-scid IL2rgnull (NSG) mice. Mice were treated with labetuzumab govitecan, h679-SN-38, or vehicle by intraperitoneal injections weekly for a total of four treatments over 28 days. By day 17 and day 24, 100% of tumors in the labetuzumab govitecan treatment arm (n = 10) and the h679-SN-38 arm (n = 9) were undetectable, respectively (Supplementary Fig. S11A). In contrast, tumors in the vehicle treatment arm demonstrated uncontrolled growth (Supplementary Fig. S11A). No significant changes in mouse weight (Supplementary Fig. S11B) or body condition score (Supplementary Fig. S11C) were observed throughout the study at the 25 mg/kg dose. Four of nine (45%) vehicle-treated mice were sacrificed prior to completion of the study as they exceeded institutional tumor size restrictions (Supplementary Fig. S11D).

We next tested labetuzumab govitecan treatment in multiple LuCaP PDxXs established from lethal mCRPC tissues (53) that express varying
levels of CEACAM5. The LuCaP 49 and LuCaP 145.1 NEPC PDXs were classified as CEACAM5low/moderate and CEACAM5high expression models, respectively, based on intensity of IHC staining (Supplementary Fig. S12). Mice were treated with labetuzumab govitecan or h679-SN-38 at 25 mg/kg or vehicle by intraperitoneal injection every 4 days. Complete responses were observed in 100% of labetuzumab govitecan (n = 10) and h679-SN-38-treated mice (n = 8) bearing LuCaP 49 PDX tumors by day 14 (Fig. 6A). Complete responses were also observed in 100% of labetuzumab govitecan-treated mice (n = 8) with LuCaP 145.1 PDX tumors by day 14, while h679-SN-38 treatment suppressed tumor growth but did not eradicate tumors in any mice (Fig. 6B). Importantly, the LuCaP 49 and LuCaP 145.1 tumor models...
were relatively resistant to cisplatin and etoposide chemotherapy (Fig. 6A and B) which is considered the standard-of-care front-line treatment for extensive stage NEPC.

In the LuCaP 49 study, the average weight loss in the labetuzumab govitecan group comparing treatment preenrollment with day 28 was 10%. However, this weight loss occurred within the first week of treatment and weights otherwise remained stable in all groups for the remainder of the study (Supplementary Fig. S13A). In addition, no significant changes in body condition scores were observed (Supplementary Fig. S13A). No significant changes in weight or body condition score were observed in mice in the LuCaP 49.1 study (Supplementary Fig. S13B). Adverse effects on liver and kidney function are often reported in association with irinotecan chemotherapy. We performed serum chemistries on days 0, 14, and 28 to assess for these and other toxicities (Supplementary Fig. S14A). Across both studies, three of 18 (17%) labetuzumab govitecan-treated mice exhibited elevated aspartate aminotransferase levels at day 28 that were less than twice the upper limit of the reference range (Supplementary Fig. S14B and S14C), indicating mild hepatotoxicity in these animals. Complete blood counts were also performed (Supplementary Fig. S14D). Across both studies, six of 18 (33%) labetuzumab govitecan-treated mice exhibited leukocytosis at day 28 (Supplementary Fig. S14E) with an increase in the neutrophil fraction (Supplementary Fig. S14F). Similar results were observed in the h679-SN-38 and cisplatin and etoposide-treated mice compared with vehicle-treated mice (Supplementary Fig. S14D).

Given the striking antitumor effects but mild toxicities associated with labetuzumab govitecan at the 25 mg/kg dose, we tested labetuzumab govitecan at a reduced dose with less frequent dosing. NSG mice bearing CEACAM5low/moderate LuCaP 49 NEPC PDX tumors or CEACAM5high LuCaP 176 ARlow/NE high PDX tumors were treated with labetuzumab govitecan or h679-SN-38 at 25 mg/kg or 12.5 mg/kg by intraperitoneal injection weekly. In the LuCaP 49 model, both dose levels of labetuzumab govitecan led to complete responses in 100% of mice (n = 7) by day 21. While both dose levels of h679-SN-38 inhibited tumor growth, only the 25 mg/kg dose led to tumor eradication (Fig. 6C). The LuCaP 176 model displayed more of a dose-dependent treatment response compared to LuCaP 49. The 25 mg/kg dose of labetuzumab govitecan led to complete responses in 100% of mice (n = 6) by day 17. In contrast, tumor eradication was observed in three of six (50%) of mice treated with 12.5 mg/kg of labetuzumab govitecan (Fig. 6D). Both dose levels of h679-SN-38 slowed tumor growth but did not diminish tumor volume. No significant changes in weight or body condition score were detected for either study (Supplementary Fig. S13C and S13D). These studies highlight the potency and efficacy of labetuzumab govitecan in CEACAM5+ prostate cancer PDX models by demonstrating that a reduced dose and administration schedule are also capable of achieving complete responses.

Discussion

The development and translation of safe and effective new therapies for NEPC are necessary to alter the course of this highly aggressive and deadly disease. The identification of tumor-restricted cell surface antigens and their targeting with antibodies, ADCs, or adoptive cell therapies has yet to make a clinical impact on the management of NEPC. Recent, substantial efforts have focused on targeting the ASCL1-regulated Notch ligand DLL3, but advanced clinical development of the promising DLL3-targeting ADC rovalpituzumab tesirine was discontinued because of excessive toxicity likely related to the pyrrolobenzodiazepine dimer payload (54). Our work indicates that CEACAM5 is a compelling cell surface antigen for therapeutic targeting in NEPC as it is expressed in over 60% of NEPC across multiple cohorts of patients, including those with end-stage disease, and demonstrates limited systemic expression. To accelerate therapeutic development, we redirected the existing CEACAM5-targeted ADC, labetuzumab govitecan, currently being evaluated for metastatic colorectal cancer, to NEPC. In multiple preclinical studies, labetuzumab govitecan treatment of patient-derived CEACAM5-expressing tumors resulted in complete responses. Labetuzumab govitecan is similar in design to the ADC sacituzumab govitecan, which was recently approved for the treatment of metastatic triple-negative breast cancer and has received fast-track designation for metastatic urothelial carcinoma and NSCLC. Labetuzumab govitecan and sacituzumab govitecan share the same unique hydrolyzable linker, as well as SN-38 as the cytotoxic payload, and have collectively demonstrated manageable toxicities in patients across several clinical studies (21, 55, 56).

Our studies examining the expression of CEACAM5 and other relevant cell surface antigens in a large cohort of lethal mCRPC samples provide significant biological insights and have important clinical implications. We identified a correlation between serum CEA levels and CEACAM5 expression in tumor tissues across a small series of patients with end-stage mCRPC, which appears most prominent in cases of NEPC. The measurement of serum CEA in the appropriate prostate cancer context (e.g., disease progression with a low PSA) might have value for diagnostic and/or therapeutic purposes in the identification, treatment selection, and disease monitoring of patients with CEACAM5+ NEPC. Further investigation of serum CEA as a biomarker in clinical trials for NEPC will be necessary to determine its utility. While expression of Trop2, PSMA, and PSCA has been reported to be relatively homogeneous in early stages of prostate cancer, our results indicate that there is significant heterogeneity in their expression in end-stage mCRPC. Our results show that CEACAM5 expression marks a biologically distinct subset of prostate cancer that has relatively minor overlap with Trop2, PSMA, or PSCA expression. The clinical implication is that CEACAM5+ NEPC will not be detected by emerging imaging modalities and may be impervious to treatment approaches directed at Trop2, PSMA, or PSCA.

We also established the functional relevance of ASCL1 and NeuroD1 expression in driving neuroendocrine lineage reprogramming of prostate cancer. These transcription factors appear to induce a simultaneous reduction in AR expression, AR-dependent NKX3-1 expression, and the acquisition of neuroendocrine differentiation and ASCL1 and NeuroD1 to the process of neuroendocrine cancer lineage reprogramming in NEPC as it is expressed in over 60% of NEPC across multiple cohorts of patients, including those with end-stage disease, and demonstrates limited systemic expression. To accelerate therapeutic development, we redirected the existing CEACAM5-targeted ADC, labetuzumab govitecan, currently being evaluated for metastatic colorectal cancer, to NEPC. In multiple preclinical studies, labetuzumab govitecan treatment of patient-derived CEACAM5-expressing tumors resulted in complete responses. Labetuzumab govitecan is similar in design to the ADC sacituzumab govitecan, which was recently approved for the treatment of metastatic triple-negative breast cancer and has received fast-track designation for metastatic urothelial carcinoma and NSCLC. Labetuzumab govitecan and sacituzumab govitecan share the same unique hydrolyzable linker, as well as SN-38 as the cytotoxic payload, and have collectively demonstrated manageable toxicities in patients across several clinical studies (21, 55, 56).

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We also established the functional relevance of ASCL1 and NeuroD1 expression in driving neuroendocrine lineage reprogramming of prostate cancer. These transcription factors appear to induce a simultaneous reduction in AR expression, AR-dependent NKX3-1 expression, and the acquisition of neuroendocrine differentiation markers. Global epigenetic reprogramming of prostate cancer induced by these pioneer transcription factors may coordinately silence the AR-enforced epithelial cancer program and engender neuroendocrine cancer programs. Studies are underway to characterize the contributions of ASCL1 and NeuroD1 to the process of neuroendocrine transdifferentiation of prostate cancer through the integration of genetic, transcriptomic, and epigenetic approaches. Our findings indicate that the biology of NEPC may parallel that of SCLC in that they share ASCL1high and NeuroD1high disease subtypes. However, whether the tuft cell variant POU2F3high or YAP1high subtypes found in SCLC (11) also exist in NEPC has yet to be determined. A recent publication suggests potential biological divergence of NEPC from SCLC in that YAP1 expression is de-enriched in NEPC compared with other subsets of mCRPC (57).

A mechanistic understanding of the regulation of CEACAM5 expression and its specificity to certain cancers has generally been lacking. Previous studies have shown that the wide-ranging modulation of cancer cell differentiation states by retinoic acid or sodium butyrate treatment impacts CEACAM5 expression (36). Our work...
demonstrates that ASCL1 promotes neuroendocrine transdifferentiation of prostate cancer which results in increased chromatin accessibility of the core promoter of CEACAM5. We suspect that this mechanism of CEACAM5 regulation by ASCL1 may be conserved in other neuroendocrine carcinomas including SCLC, but additional functional studies will be necessary for confirmation. An interesting question arising from our findings is whether additional pioneer transcription factors may similarly modulate the epigenomes of other tumor types to permit CEACAM5 expression in nonneuroendocrine cancer cell contexts.

The diversity of prostate cancer phenotypes that emerge with castration resistance and their coexistence in late-stage patients indicate that single-targeted therapies may be ineffective. The existence of multiple subtypes of NEPC that may affect expression of target antigens like CEACAM5 and DL3 in NEPC further compound this issue. Targeted prostate cancer therapies with multiple mechanisms of action or combinations of treatments may be necessary to conquer such diversity. Our in vivo studies demonstrate strong antitumor activity of labetuzumab govitecan and, to a lesser extent, the nonspecific h679-SN-38 ADC which is likely a consequence of linker hydrolysis and systemic release of SN-38. Labetuzumab govitecan therefore represents a monotherapy that delivers both regional, antigen-specific and systemic, nonspecific tumor killing. The benefit of a moderately stable ADC linker may be increased efficacy in patients with intratumoral and intratumoral heterogeneity, such as that observed in cases of mixed NEPC which occurs in up to 50% of cases. This bystander effect has also been demonstrated in a number of other tumor types for the sister molecule sacituzumab govitecan (58, 59).

The results of these studies have led to planning for a forthcoming phase I/II clinical trial of labetuzumab govitecan for patients with CEACAM5− NEPC. CEACAM5 is also expressed in other neuroendocrine carcinomas including SCLC and MTC. More than half of SCLC are ASCL1hi (11) with the majority expressing CEACAM5, while advanced MTC are almost uniformly ASCL1hi and express CEACAM5 (38). Investigation of whether labetuzumab govitecan is effective in these and other CEACAM5+ neuroendocrine carcinomas may also be warranted.

Authors’ Disclosures

D.C. DeLucia reports other from Immunomedics Inc. during the conduct of the study; T.M. Cardillo reports other from Immunomedics, Inc. during the conduct of the study; other from Immunomedics, Inc. outside the submitted work. E. Corey reports grants from Pacific Northwest Prostate Cancer SPORE (P50CA97186), NCI P01 CA163227, and the Department of Defense Prostate Cancer Biorepository Network (W81XWH-14-2-0183) during the conduct of the study; L.D. True reports P01 CA163227, and the Department of Defense Prostate Cancer Biorepository Network (W81XWH-14-2-0183) during the conduct of the study; personal fees from Immunomedics during the conduct of the study; other from Immunomedics, Inc. outside the submitted work. L.D. True reports P01 CA163227, and the Department of Defense Prostate Cancer Biorepository Network (W81XWH-14-2-0183) during the conduct of the study. L.D. True reports P01 CA163227, and the Department of Defense Prostate Cancer Biorepository Network (W81XWH-14-2-0183) during the conduct of the study; personal fees from Immunomedics, Inc. outside the submitted work. E. Corey reports grants and nonfinancial support from Immunomedics, Inc. during the conduct of the study. No disclosures were reported by the other authors.

Authors’ Contributions

D.C. DeLucia: Conceptualization, data curation, formal analysis, validation, investigation, visualization, methodology, writing-original draft, writing-review and editing. T.M. Cardillo: Resources, writing-review and editing. L. Ang: Investigation. M.P. Labrecque: Resources. A. Zhang: Data curation, formal analysis, investigation, methodology. J.E. Hopkins: Data curation, formal analysis, investigation. N. De Sarkar: Data curation, formal analysis. I. Coleman: Data curation, formal analysis. R.M. Gil da Costa: Resources. E. Corey: Resources. L.D. True: Resources. M.C. Haffner: Resources. M.T. Schweizer: Resources. C. Morrissey: Resources. P.S. Nelson: Resources, supervision, writing-review and editing. J.K. Lee: Conceptualization, data curation, formal analysis, supervision, funding acquisition, investigation, visualization, methodology, writing-original draft, project administration, writing-review and editing.

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Regulation of CEACAM5 and Therapeutic Efficacy of an Anti-CEACAM5–SN38 Antibody–drug Conjugate in Neuroendocrine Prostate Cancer

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