Preclinical Characterization of Human Kallikrein 2 (hK2) as a Novel Target for the Treatment of Prostate Cancer

Fei Shen[1], William Kevin Kelly[2], Neeta Pandit-Taskar[3,4], Theresa McDevitt[1], Ryan Smith[1], Krista Menard[1], Kathryn Packman[1], Ruchi Chaudhary[1], Brent Rupnow[1], Margaret Yu[1], Mary Guckert[1], Ken Tian[1], Gerald Chu[1], Hong Xie[1], Daniel Patricia[1], Steven Max[1], Michael J. Morris[5,6]

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KEY TAKEAWAYS

hK2 is a highly prostate-specific surface target that is expressed across different stages of prostate cancer. Targeting hK2 with multiple therapeutic modalities demonstrated confirmed antitumor activity in preclinical models

hK2, human kallikrein 2.

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CONCLUSIONS

- hK2 was expressed across PC stages. Its expression is largely maintained in mCRPC post ARSI treatment, with increased heterogeneity compared with LPC and mCSPC
- hK2 demonstrated cell surface expression with internalization upon antibody binding
- Antitumor activity was observed across multiple hK2-targeting agents in PC mouse models, with confirmed hK2 targeting to mCRPC tumors in patients with no off-tumor targeting

hK2, human kallikrein 2; LPC, localized prostate cancer; mCSPC, metastatic castration-sensitive prostate cancer; mCRPC, metastatic castration-resistant prostate cancer; PC, prostate cancer.

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INTRODUCTION

- Of the available treatment options for patients with metastatic castration-resistant prostate cancer (mCRPC), few are durably effective, highlighting a continued unmet need for novel drug development[1,2]
- Human kallikrein 2 (hK2), encoded by the KLK2 gene, is a trypsin-like androgen-regulated protease, which is overexpressed in PC and has little to no expression in nonprostate tissues[3,4]
- Multiple first-in-human studies are investigating hK2 as a novel target in mCRPC[5-7]
- We used a series of preclinical experiments to evaluate hK2 as a therapeutic target for PC

OBJECTIVES:

- To characterize hK2 expression in different stages of PC
- To characterize hK2-targeting therapeutics in the preclinical setting and to evaluate hK2 as a therapeutic target for PC

hK2, human kallikrein 2; mCRPC, metastatic castration-resistant prostate cancer; PC, prostate cancer.

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METHODS

hK2 expression

- hK2 expression was assessed by immunohistochemistry (IHC) in non-metastatic castration-sensitive PC (LPC; n = 100), metastatic castration-sensitive PC (mCSPC; n = 98), and mCRPC (n = 45) samples obtained from tumor banks and warm autopsy tumor microarrays
  - The deparaffinization, antigen retrieval, and immunostaining of samples were conducted on a Bond RX automated stainer (Leica)
- Immunostained controls and samples were scanned on an Aperio AT2 scanner (Leica) scanner and reviewed for qualitative analysis by image capture
- Multiplex immunofluorescence (mIF) was used to assess hK2 expression at different mCRPC tumor sites. The mIF assay iteratively stains, images, and performs advanced image analysis for marker expression at the single-cell level. Pathology review of the mCRPC samples confirmed the region of interest. hK2 staining was required to be co-localized on epithelial origin cells by cytokeratin expression. Analysis of the position of staining relative to individual tumor cells was then extrapolated through image analysis
- hK2 cell surface expression in a human prostate cancer cell model (VCaP) and fresh mCRPC patient tumor samples was assessed by flow cytometry using an anti-hK2 antibody
- hK2-targeting antibody internalization over time was visualized by confocal IF microscopy in VCaP cells

hK2, human kallikrein 2; IHC, immunohistochemistry; LPC, localized prostate cancer; mCRPC, metastatic castration-resistant prostate cancer; mCSPC, metastatic castration-sensitive prostate cancer; mIF, multiplex immunofluorescence; PC, prostate cancer; VCaP, vertebral-cancer of the prostate.

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METHODS

hK2 antitumor activity

- VCaP xenograft models were used to evaluate antitumor activity, defined as the difference between the mean tumor volume of the treatment and control groups (% ΔTGI)
  - Immune-compromised male mice were injected subcutaneously (SC) with 1×10^7 VCaP cells
  - Treatment with a single dose of actinium-225 (^{225}Ac)-labeled hK2-targeting antibody or anti-hK2 chimeric antigen receptor (CAR) T-cell therapy was initiated after SC tumors were established. The bispecific hK2xCD3 antibody was administered twice weekly after in vitro expanded human pan T cells were injected (2×10^7)
  - Tumor volume was measured twice weekly. Each in vivo experiment was done independently

mCRPC tumor uptake of indium-111 (^{111}In)-labeled anti-hK2 antibody

- Whole-body planar and single-photon emission computed tomography/computed tomography (SPECT/CT) imaging were used to assess hK2-targeting antibody tumor uptake in patients with mCRPC, with tumor location verification by of gallium-68 (^{68}Ga) prostate-specific membrane antigen (PSMA) positron emission tomography (PET)/CT

^{68}Ga, gallium-68; ^{225}Ac, actinium-225; CAR-T, chimeric antigen receptor T-cell; CD3, cluster of differentiation 3; CT, computed tomography; hK2, human kallikrein 2; mCRPC, metastatic castration-resistant prostate cancer; PET, positron emission tomography; PSMA, prostate-specific membrane antigen; SC, subcutaneous; SPECT, single-photon emission computed tomography; TGI, tumor growth inhibition; VCaP, vertebral-cancer of the prostate.

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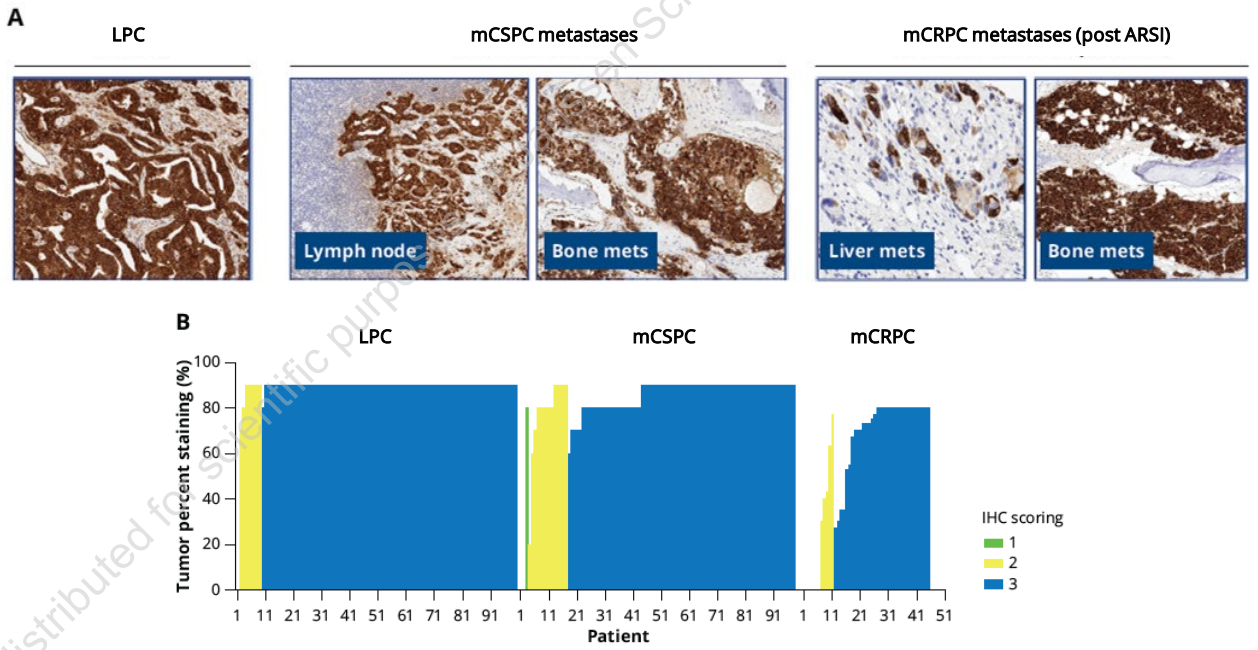
RESULTS

Evaluation of hK2 expression across different stages and tumor sites of PC tissue

- IHC confirmed robust hK2 expression across different PC stages with homogenous expression in LPC and mCSPC and increased heterogeneity in mCRPC (A)
- Of the patient tumor samples tested, 91/100, 81/98, and 34/45 patients with LPC, mCSPC, and mCRPC, respectively, had high tumor expression of hK2 (IHC staining intensity score = 3) (B)



ARSI, androgen receptor signaling inhibitor; hK2, human kallikrein 2; IHC, immunohistochemistry; LPC, localized prostate cancer; mCRPC, metastatic castration-resistant prostate cancer; mCSPC, metastatic castration-sensitive prostate cancer; mets, metastases; NHT, novel hormonal therapy; prostate cancer.

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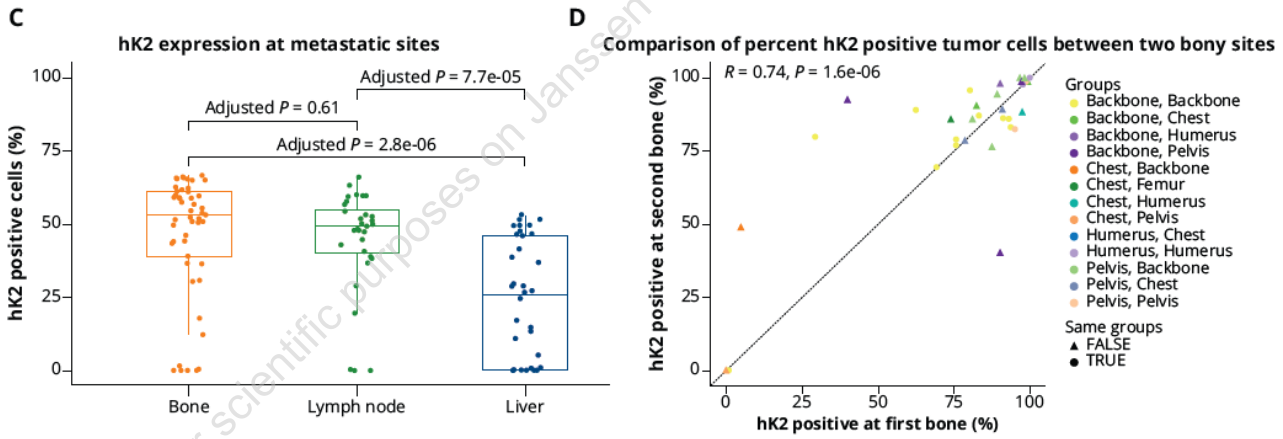
RESULTS

Evaluation of hK2 expression across different stages and tumor sites of PC tissue

- hK2 was commonly expressed in bone and lymph node metastases (C)
  - Mean percentage tumor staining of hK2 in mCRPC samples was 67.6% (bone), 65.7% (lymph nodes), and 33.5% (liver) by mIF
- Correlation of hK2 expression across mCRPC bony lesions (R = 0.74, P < 1.6e-06) was observed, demonstrating intrapatient and interlesion homogeneity in bone lesions (D)



hK2, human kallikrein 2; mCRPC, metastatic castration-resistant prostate cancer; mIF, multiplex immunofluorescence; PC, prostate cancer.

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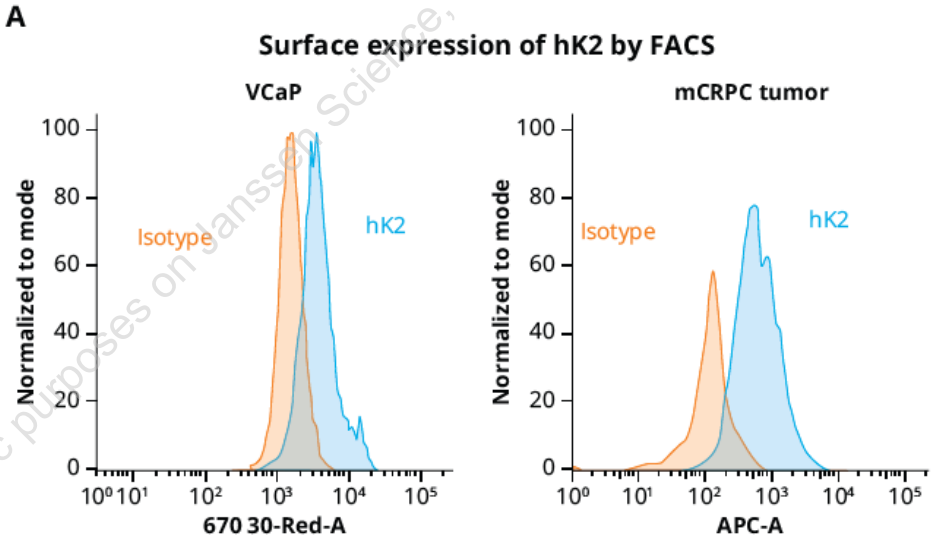
RESULTS

hK2 cellular localization and hK2-targeting antibody internalization

- Flow cytometry confirmed hK2 cell surface expression in both VCaP cells and freshly dissociated mCRPC tumor samples (A)



FACS, fluorescence-activated cell sorting; hK2, human kallikrein 2; mCRPC, metastatic castration-resistant prostate cancer; VCaP, vertebral-cancer of the prostate.

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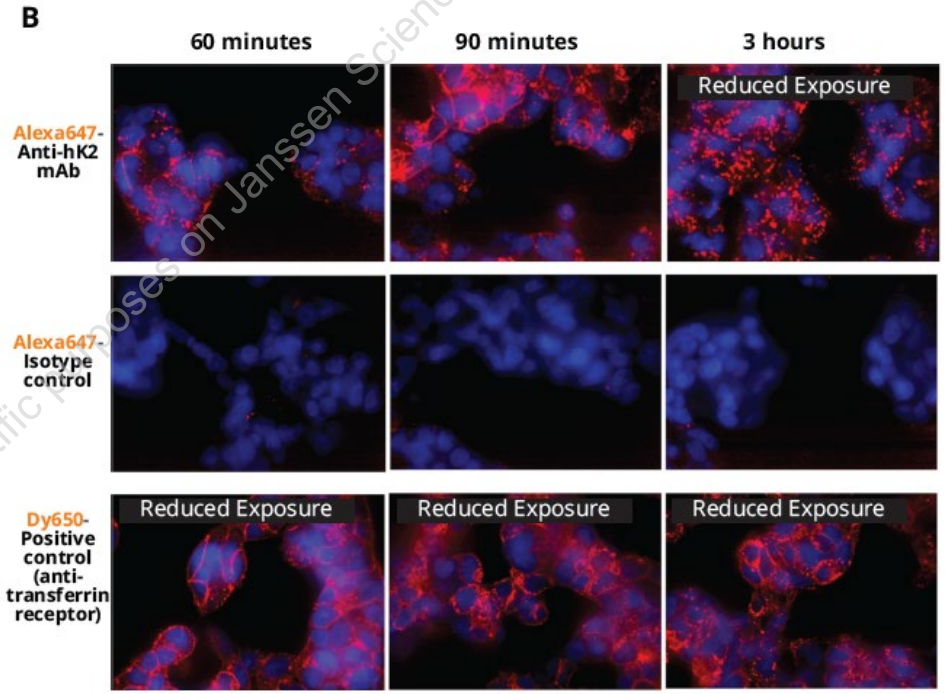
RESULTS

hK2 cellular localization and hK2-targeting antibody internalization

- Confocal microscopy demonstrated hK2 cell surface expression in VCaP cells, with rapid antibody internalization within 3 hours (B), observed as increased intracellular punctae formation



hK2, human kallikrein 2; mAb, monoclonal antibody; VCaP, vertebral-cancer of the prostate.

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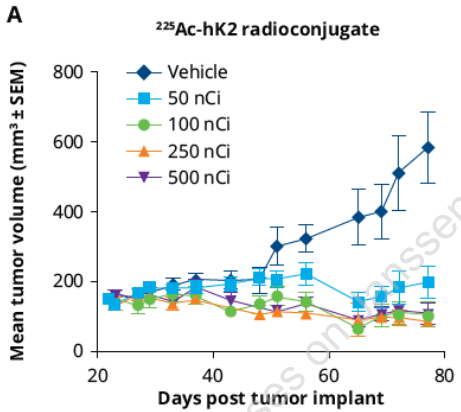
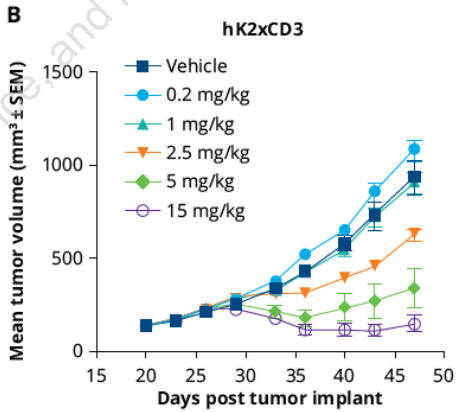
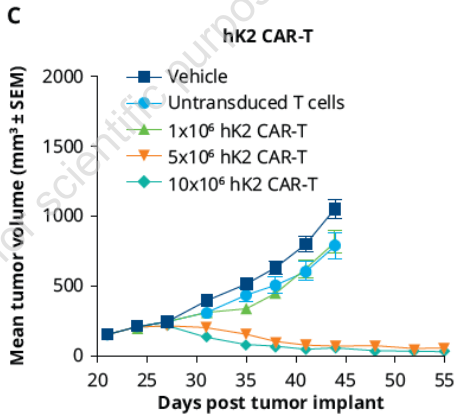
RESULTS

Proof of concept of hK2 as a therapeutic target with 3 novel hK2-targeting agents in preclinical PC mouse models

- Robust antitumor activity was observed following treatment with each hK2-targeting agent in the xenograft mouse model (A, B, C)
  - Treatment with ^{225}Ac-labeled hK2-targeting antibody led to reduced tumor growth across doses, with a ΔTGI of 110% at 500 nCi compared with control (A and Table)
  - Treatment with a bispecific antibody targeting CD3 on T cells and hK2 on tumor cells led to reduced tumor growth with increasing doses, with ΔTGI of 99% at 15 mg/kg compared with control (B and Table).
  - Treatment with the anti-hK2 CAR T-cell therapy led to reduced tumor growth at higher doses, with ΔTGI of 110% at 10 x 10^6 CAR T-cells compared with control (C and Table)







Agent	ΔTGI at the highest dose level (compared with control)
hK2 radioconjugate (^{225}Ac)	110% at 500 nCi
hK2xCD3	99% at 15 mg/kg
hK2 CAR-T	110% at 10x10^6 CAR T-cells

^{225}Ac, actinium-225; CAR-T, chimeric antigen receptor T-cell; CD3, cluster of differentiation 3; hK2, human kallikrein 2; SEM, standard error of the mean.

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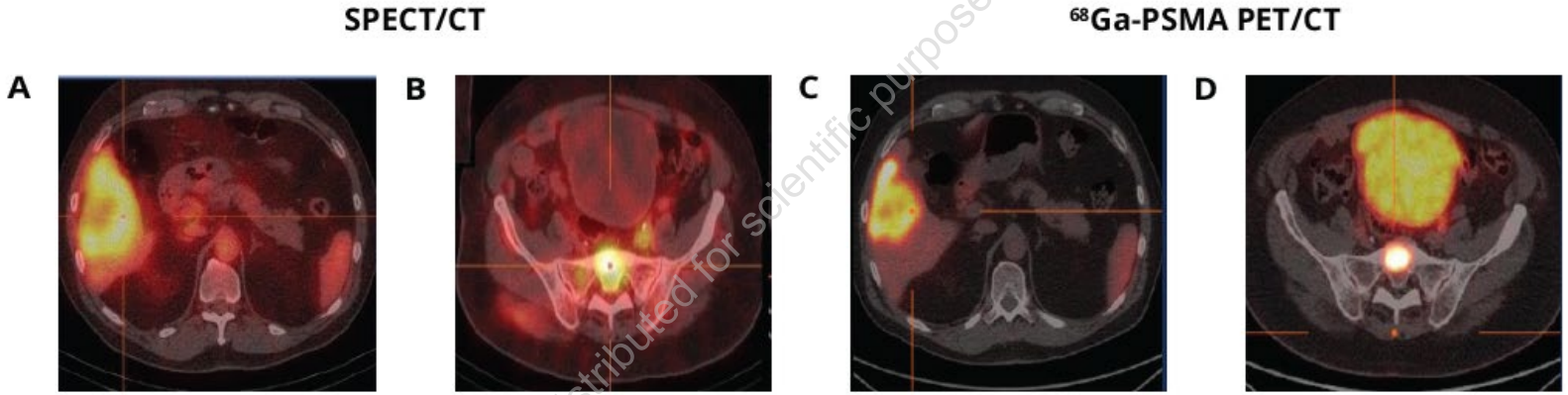
RESULTS

Confirmed tumor uptake of an anti-hK2 antibody in patients with mCRPC

- SPECT/CT scans confirmed accumulation of the hK2-targeting antibody to PC tumors with no off-target uptake in patients with mCRPC (A, B)
- Tumor targeting was confirmed by 68Ga-PSMA PET/CT (C, D)



^{68}Ga, gallium-68; CT, computed tomography; hK2, human kallikrein 2; mCRPC, metastatic castration-resistant prostate cancer; PET, positron emission tomography; PSMA, prostate-specific membrane antigen; SPECT, single-photon emission computed tomography.

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DISCLOSURES:

Fei Shen declares no competing financial interests. Neeta Pandit-Taskar: Honoraria - Actinium Pharmaceuticals; Consulting or Advisory Role - Illumina, Progenics, Telix, Lantheus; Speakers' Bureau - Actinium Pharmaceuticals, Telix; Research Funding (institutional) - Bayer Health, Bristol-Myers Squibb, Clarity Pharmaceuticals, Imaginab, Janssen, Regeneron, Ymabs, Innervate; Travel, Accommodations, Expenses - AstraZeneca, Bayer. Michael J Morris: Stock and Other Ownership Interests – Doximity; Consulting or Advisory Role - Lantheus Medical Imaging, AstraZeneca, Amgen, Daiichi, Convergent Therapeutics, Pfizer, ITM Isotope Technologies Munich, Clarity Pharmaceuticals, Blue Earth Diagnostics, POINT Biopharma, Telix Pharmaceuticals, Progenics, Z-Alpha; Research Funding - Bayer (Inst), Progenics (Inst), Corcept Therapeutics (Inst), Roche/Genentech (Inst), Janssen (Inst), Celgene (Inst), Novartis (Inst), Astellas Pharma (Inst); Travel, Accommodations, Expenses - AstraZeneca, APCCC, Memorial Sloan Kettering Cancer Center.

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