EM1031, a novel KLK2 x CD3 bispecific T-cell engager with highly effective efficacy in preclinical models

Abstract #3520

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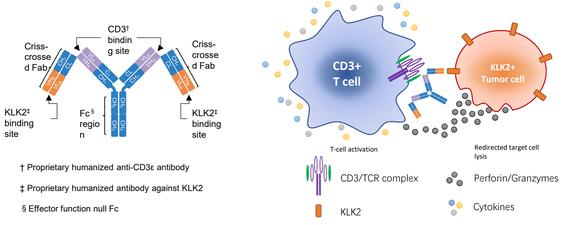
Background

Human kallikrein 2 (KLK2) is a membrane-bound trypsin-like serine protease that shares 78% homology with prostate specific antigen (PSA)[1,2]. Directly regulated by androgen receptor (AR)[3,4], KLK2 is specifically expressed in epithelial cells of prostate and prostate cancer cells, which makes it a promising tumor associated target.

EM1031 is a novel KLK2 x CD3 bispecific T-cell engager (TCE) based on EpimAb's proprietary CD3 antibody and bispecific antibody platform, adopting optimized CD3 affinity and binding arm valency.

For reference, Bi2023-4a is a clinical stage TCE('1+1' format, ~125kD), consisting of an anti-KLK2 Fab and an anti-CD3 scFv.



Results

KLK2 Is Closely Associated With Prostate Cancer Cells

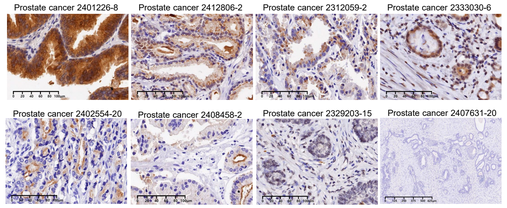


Figure 1. Evaluation of KLK2 expression in primary prostate cancer samples by using immunohistochemistry (IHC)

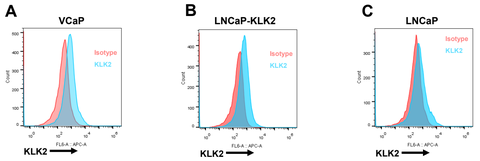


Figure 2. KLK2 expression on the cell surface of prostate cancer cell lines. (A) VCaP (ATCC, CRL-2876); **(B)** LNCaP-KLK2, a human KLK2 over expressed prostate cancer cell line **(C)** LNCaP (ATCC, CRL-1740).

EM1031 Binds to KLK2 With High Affinity and Specificity

	Target	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_D (M)
EM1031	KLK2	1.45E+06	4.21E-03	2.91E-09
	KLK3	N/A	N/A	No binding
	CD3E	2.73E+06	1.48E-02	5.41E-09

Table 1. Target binding affinities of EM1031 were measured using grating-coupled interferometry (GCI).

EM1031 Does Not Activate T Cells in the Absence of Target Cells *in vitro*

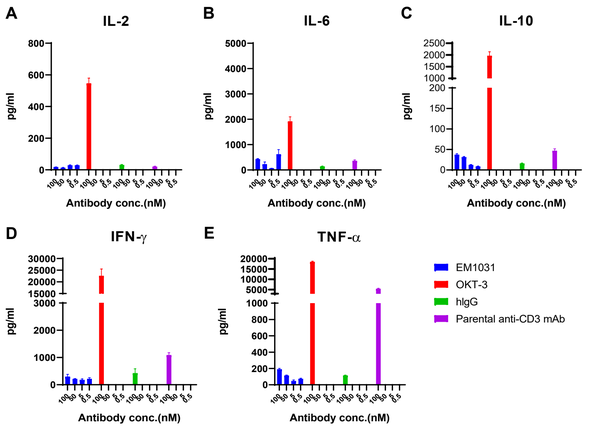


Figure 3. Cytokine release induced by EM1031 in high density PBMC *assay.* High density human PBMC treated by antibodies for 24 hours, followed by measuring of (A) IL-2, (B) IL-6, (C) IL-10, (D) IFNγ and (E) TNFα.

EM1031 Showed Potent *In Vitro* Efficacy

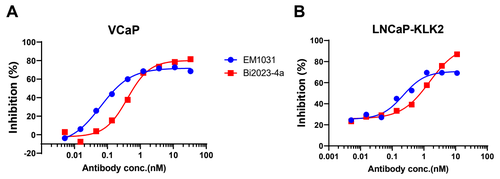


Figure 4. *In vitro* redirected T-cell cytotoxicity assay with prostate cancer cells. Human T cells were cocultured with KLK2+ tumor cell lines **(A)** VCaP, **(B)** LNCaP-KLK2 and treated with T-cell for 48 hours. Tumor cell lysis was measured using CellTiter-Glo.

EM1031 Induced Less Cytokine Release in Co-Cultured Redirected Cytotoxicity Assays

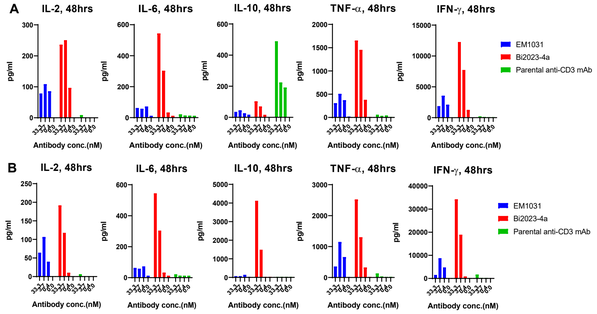


Figure 5. Cytokine release levels in the *in vitro* co-cultured redirected cytotoxicity assays. Human T cells were cocultured with (A) VCaP and (B) LNCaP-KLK2, respectively. Supernatants were collected at 48 hours for the measurement of IL-2, IL-6, IL-10, TNFα and IFNγ concentrations.

EM1031 Showed Robust *In Vivo* Anti-Tumor Efficacy in Prostate Cancer Models

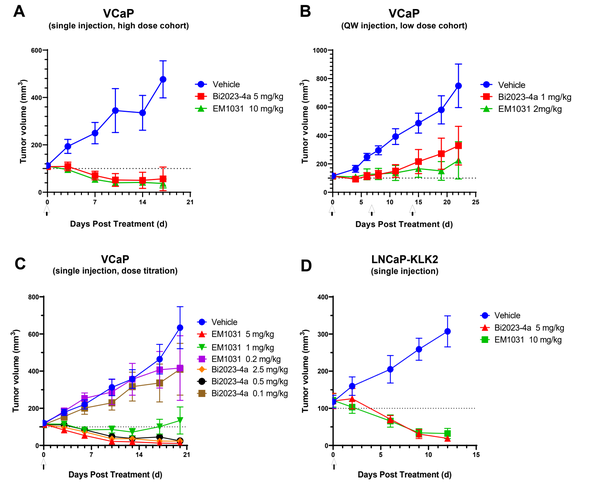


Figure 6. *In vivo* efficacy study in prostate cancer cell line derived xenograft (CDX) models. NCG mice were engrafted with human PBMC via i.p. injection and inoculated with tumor cells subcutaneously. Animals were randomly grouped and treated with test articles when the tumor size reached 100-150 mm³. Test articles were administered (i.p.) as indicated by arrows. **(A)** VCaP CDX model with higher dosages; **(B)** VCaP CDX model with lower dosages; **(C)** VCaP CDX model with dosages titration; **(D)** LNCaP-KLK2 CDX model.

EM1031 Demonstrated a Better Safety Profile in Mice

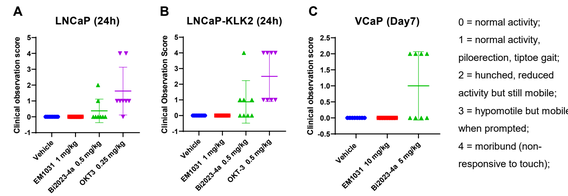


Figure 7. Clinical observation of prostate cancer CDX model treated with different articles. NCG mice were engrafted with human PBMC via i.p. injection and inoculated with tumor cells subcutaneously. Animals were randomly grouped and treated with test articles when the tumor size reached 100-150 mm³. Test articles were administered (i.p.) by single dose and clinical observation in **(A)** LNCaP CDX model at 24h, (B) LNCaP-KLK2 CDX model at 24h and (C) VCaP CDX model at Day7.

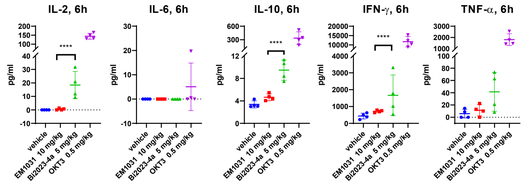


Figure 8. *In vivo* cytokine release in VCaP CDX model. NCG mice were engrafted with human PBMC via i.p. injection and inoculated with VCaP tumor cells subcutaneously. Animals were randomly grouped and treated with test articles when the tumor size reached 100-150 mm³. Test articles were administered (i.p.) and serum were collected at 6 hours for the measurement of IL-2, IL-6, IL-10, IFNγ and TNFα.

Conclusion

- KLK2 is a promising tumor associated target for prostate cancer due to its tissue specific expression and localization on prostate cancer cells surface.
- EM1031 is a KLK2 targeting TCE generated with the clinically validated CD3 antibody panel and tetravalent FIT-Ig platform[5].
- EM1031 candidate showed potent preclinical efficacy and differentiated safety profile by inducing less cytokine release compared to a clinical benchmark.

References:
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3. Lawrence et al. *Endocrine reviews* vol. 31,4 (2010): 407-46.
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