An HSV-1-based vector for local delivery of IL-12 and IL-2 reshapes the immune landscape leading to tumor clearance and systemic immune surveillance

Dana M. Previte, Ph.D.[1]; Meghan M. Conner, Ph.D.[1]; Trevor J. Parry, Ph.D.[1]; Suma M. Krishnan, M.S.[1]

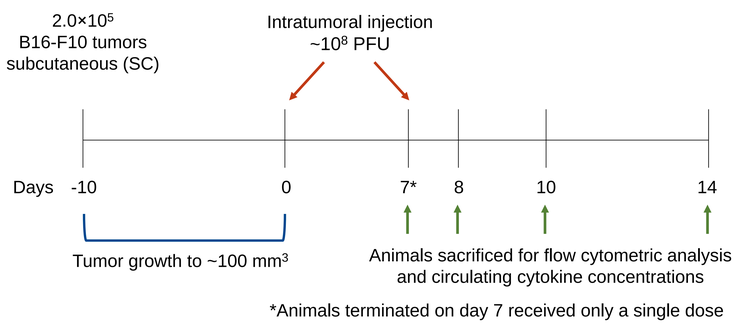
[1]Krystal Biotech, Inc., Pittsburgh, Pennsylvania

AACR 2024 Annual Meeting
POSTER #7260

Introduction

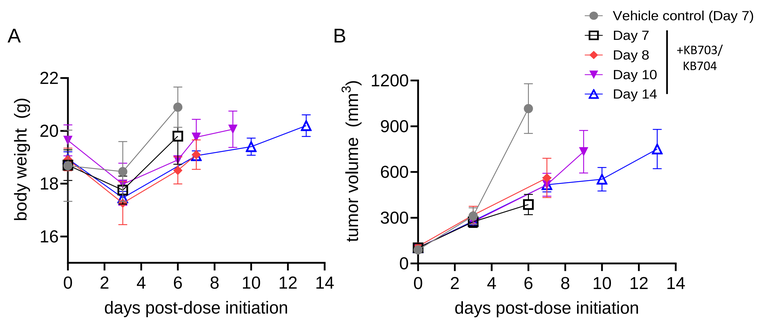
- Clinical use of recombinant interleukin (IL)-12 and -2 has been hindered due to unfavorable kinetics and toxicity associated with systemic exposure.
- KB707, a replication-defective herpes simplex virus type 1 (HSV-1)-based vector encoding human IL-12 and -2, has been developed for localized treatment of solid tumors, with clinical trials ongoing for intratumoral (NCT05970497) and inhaled (NCT06228326) administration.
  - Prior studies in mice demonstrated that local vector-encoded cytokine administration enhanced murine IL-12/IL-2 expression while limiting systemic exposure compared to conventional recombinant protein therapy.
  - In the K7M2 osteosarcoma lung metastasis mouse model, intratracheal administration of KB707-surrogate vectors KB703/KB704 (encoding murine IL-12/-2, respectively) resulted in enhanced animal survival and delayed tumor recurrence in a rechallenge model.
  - In the B16-F10 melanoma mouse model, intratumoral KB703/KB704 treatment led to improved survival in a unilateral tumor model, an abscopal effect in a bilateral tumor model, and protected against tumor recurrence in a rechallenge model.
  - Together, these results suggested that vector-mediated delivery of IL-12 and -2 could elicit both local and systemic anti-tumor immune responses.
- This study aimed to elucidate the underlying changes to the immune landscape in B16-F10 tumor-bearing animals treated intratumorally with murine surrogate vectors KB703/KB704.

Experimental design



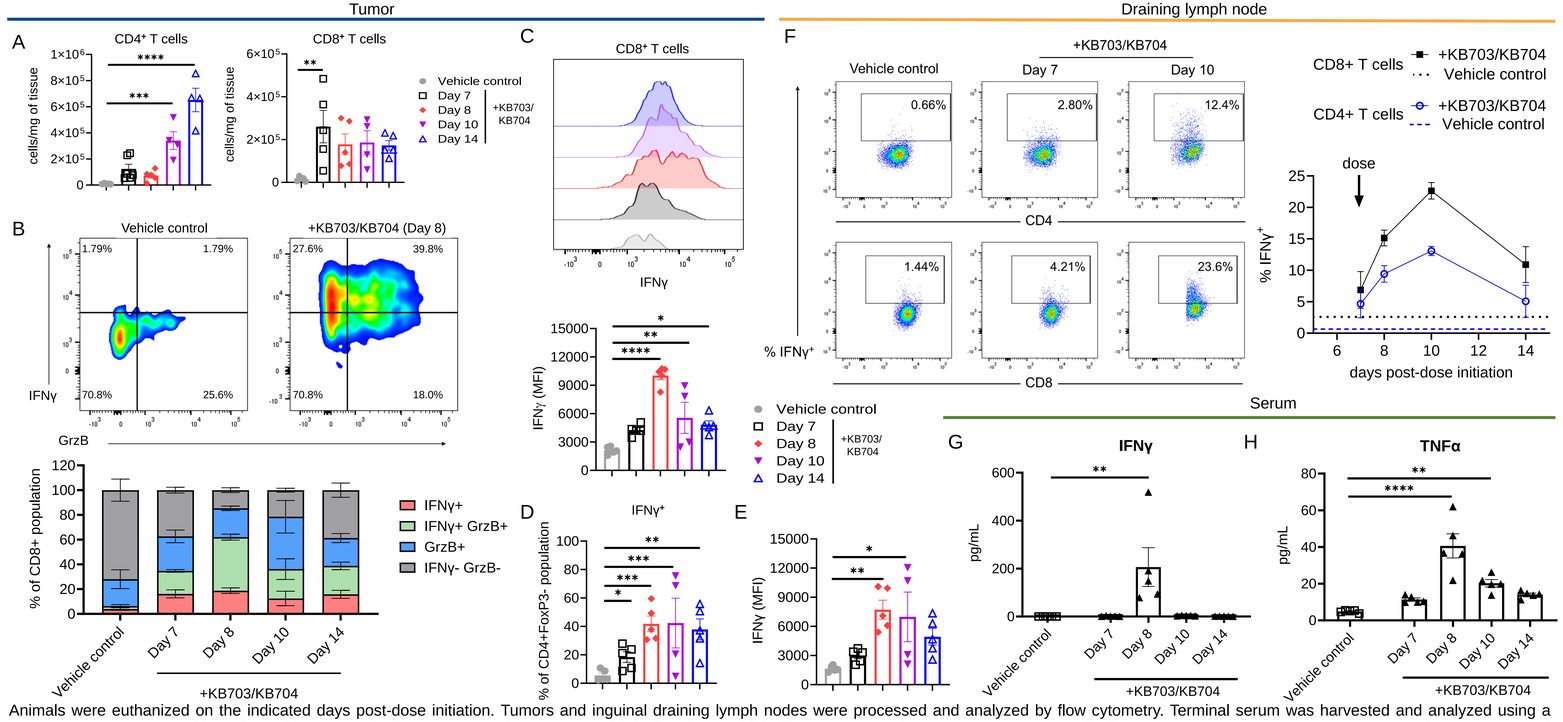
*Animals terminated on day 7 received only a single dose

Intratumoral injection of KB703/KB704 inhibits B16-F10 tumor growth



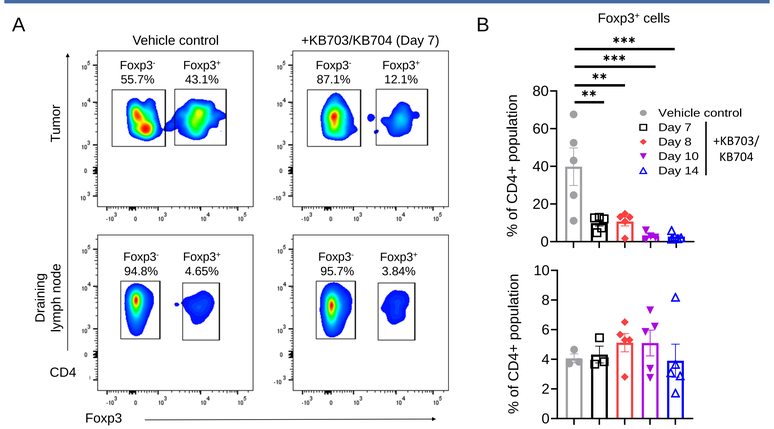
A. Body weights and **B.** tumor volumes of vehicle- and KB703/KB704-treated mice. Groups are indicated by the day of termination. Data are displayed as means ± standard error of the mean (SEM) of n=5 animals per group.

Intratumoral KB703/KB704 treatment enhances local and systemic CD8⁺ and CD4⁺ T cell effector responses



Animals were euthanized on the indicated days post-dose initiation. Tumors and inguinal draining lymph nodes were processed and analyzed by flow cytometry. Terminal serum was harvested and analyzed using a multiplex cytokine panel (Meso Scale Discovery). **A.** Total numbers of intratumoral CD4⁺ Foxp3⁻ and CD8⁺ T cells per mg of harvested tumor tissue. **B.** Representative flow plots (upper) and quantification (lower) of interferon-γ (IFNγ) and granzyme B (GrzB) expression by intratumoral CD8⁺ T cells. **C.** Representative histograms (upper) and quantification (lower) of IFNγ expression by intratumoral CD8⁺ T cells (MFI – mean fluorescence intensity). **D.** Percentage of intratumoral IFNγ⁺ cells. **E.** Quantification of IFNγ expression by intratumoral CD4⁺ Foxp3⁻ T cells. **F.** Representative flow plots (left) and percentages (right) of IFNγ⁺ CD4⁺ or CD8⁺ T cells in tumor draining lymph node. **G-H**. Serum concentrations of (**G.**) IFNγ and (**H.**) tumor necrosis factor-α (TNFα). Data are displayed as means ± SEM of n=3-5 animals per group. Statistical significance was determined using a one-way ANOVA with Dunnett's multiple comparisons test to compare each vector-treated group to the vehicle control where appropriate. *=p<0.05; **=p<0.01;***=p<0.001; ****=p<0.0001.

KB703/KB704-driven cytokine expression in the tumor does not enhance the frequency of regulatory T cells



A. Representative flow plots depicting frequency of Foxp3⁺ (regulatory) and Foxp3⁻ (conventional) CD4⁺ T cells in the tumor (top panels) and draining lymph node (bottom panels). **B.** Percent Foxp3⁺ cells of total CD4⁺ T cell population in the tumor (top panel) and draining lymph node (bottom panel). Statistical significance was determined using a one-way ANOVA with Dunnett's multiple comparisons test to compare each vector-treated group to the vehicle control. **=p<0.01; ***=p<0.001.

Conclusions

- Consistent with prior studies, local vector-driven expression of IL-12 and IL-2 slows the progression of checkpoint inhibitor-refractory B16-F10 melanoma tumors.
- Expression of IL-12 and IL-2 in the tumor microenvironment results in increased total numbers of tumor-infiltrating CD8⁺ and CD4⁺ T cells.
  - Additionally, KB703/KB704 treatment enhanced the frequency of IFNγ-expressing T cells both in the tumor and tumor-draining lymph node, leading to higher levels of circulating proinflammatory cytokines (IFNγ and TNFα).
- Exogenous full-length IL-2 does not increase regulatory T cell frequencies in the tumor or draining lymph node when co-expressed with IL-12.

Acknowledgements/Disclosures

These studies were funded by Krystal Biotech, Inc.

Krystal Biotech, Inc. would like to thank Crown Bioscience for their contributions to the work presented here. All animal studies were performed in an AAALAC accredited facility, and protocols were IACUC approved prior to initiation.

All authors are current employees of Krystal Biotech, Inc.