A replication-defective, HSV-1 based gene therapy for localized delivery of combinatorial Interleukins-12 and -2 for the treatment of cutaneous malignancies

Dana M. Previte, Ph.D.[1]; Mary Jane Duermeyer, B.S.[1]; Jorge Guzman Lepe, M.D.[1]; Trevor J. Parry, Ph.D.[1]; Suma M. Krishnan, M.S.[1]

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

Krystal

SITC 2023
Annual Meeting
POSTER #1065

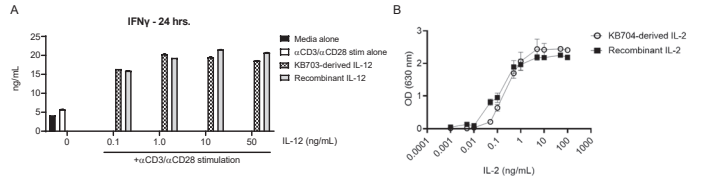
Abstract

Background: Interleukin(IL)s-12 and -2 are recognized as potent anti-tumor molecules; yet, balancing effective dosing while mitigating systemic toxicity presents a significant hurdle for their use clinically. A targeted delivery system that provides sustained local cytokine levels in the tumor microenvironment, while minimizing systemic exposure and its associated toxicities, may effectively tip the balance to overcome the recognized limitations of IL-12 and -2 therapies. Krystal Biotech, Inc. has developed KB707, a replication-defective herpes simplex virus type 1 (HSV-1)-derived vector encoding human IL-12 and -2, for redosable treatment of solid tumors.

Methods: As the human cytokines are only partially cross-reactive in mice, surrogate vectors were constructed to express murine *Il12* and *Il2*, termed KB703 and KB704, respectively, for nonclinical development. For efficacy studies, C57BL/6 mice were inoculated with B16F10 tumors, a checkpoint inhibitor-refractory melanoma line, subcutaneously on day 0, and cohorts were treated by intratumoral injection with vehicle, single, or combined vectors.

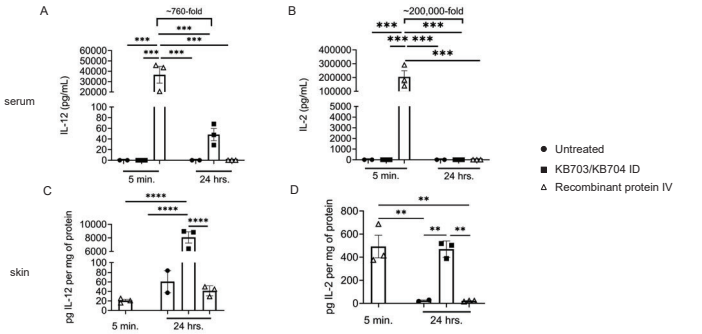
Results: Systemic cytokine exposure was limited with vector treatment compared to clinically relevant doses of intravenous recombinant proteins. With respect to efficacy studies, all control animals succumbed to tumor burden by day 31; combined KB703/KB704 therapy resulted in a significant improvement in survival by day 70 and had the highest survival rate of all treatment groups. In a rechallenge study, a subgroup of KB703/KB704 intratumorally dosed animals were reinoculated with B16F10 tumors 55 days post-initial inoculation. >50% of these animals survived to the study's endpoint, 45 days post-rechallenge, without additional intervention. These results suggest that vector-derived IL-12 and -2 treatment induces a durable anti-tumor memory response. To test the robustness of this approach, a bilateral tumor model was employed where animals were inoculated with primary B16F10 tumors on day 0 and secondary tumors at a distal site on day 0, 4, or 10. Primary tumors were treated with either vehicle control or KB703/KB704. Vector treatment resulted in at least some degree of secondary (untreated) tumor growth inhibition, suggesting an abscopal effect, the magnitude of which was directly proportional to the interval between primary and secondary tumor instillation.

Figure 1. Vector-derived murine IL-12 and IL-2 demonstrate equivalent bioactivity to commercially available recombinant proteins



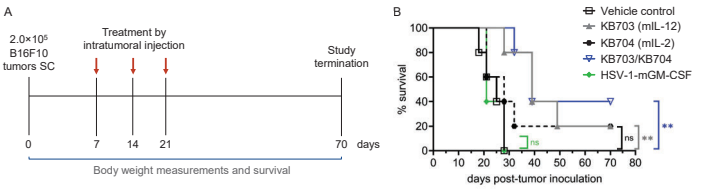
HEK 293FT cells were transduced with either KB703 (mIL-12) or KB704 (mIL-2) for 24-hours at a multiplicity of infection of 1. Supernatants were collected and cytokine concentrations were determined by ELISA (R&D systems). These supernatants served as the respective sources of vector-derived cytokines.
A. Splenocytes were isolated from naïve BALB/c mice and co-cultured with either media alone or αCD3/αCD28-coated beads (Invitrogen) to induce stimulation and Interferon (IFN)γ secretion. IL-12, from either vector-derived supernatants or recombinant protein (R&D Systems), was titrated into splenocyte cultures at the indicated concentrations. Cultures were incubated for 24-hours and supernatants were harvested for IFNγ ELISA (BioLegend). Data are displayed as means ± standard deviation (SD) of samples assayed in duplicate. **B.** HEK-Blue™ IL-2 reporter cells (Invivogen) were cultured with either vector-derived or recombinant IL-2 protein at the indicated concentrations for 24-hours. Supernatants were harvested and assayed for SEAP activity as per manufacturer's instructions. Data are displayed as means ± SD of triplicate wells.

Figure 2. Vector-mediated delivery of IL-12 and IL-2 minimizes systemic cytokine exposure while enhancing local effector concentrations as compared to recombinant proteins administered intravenously at clinically-relevant doses



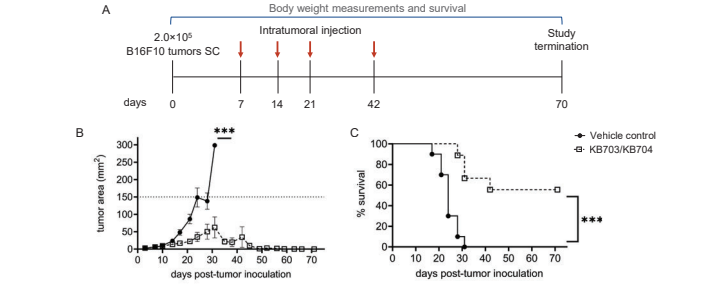
C57BL/6 mice were treated with KB703/KB704 via intradermal (ID) injection (~10^8 total PFU) or IL-12 and IL-2 recombinant protein (125 ng and 600 ng, respectively) intravenously (IV) at murine equivalent doses to human IL-12 and IL-2 that demonstrated clinical efficacy and toxicity in humans[1-3]. Untreated animals served as negative controls. **A-B.** Serum was sampled at 5 minutes and 24-hours post-article administration to compare peak systemic cytokine exposure for recombinant protein and vector treatment, respectively, and assayed by ELISA. Values indicate the difference in magnitude between peak exposure (5 minutes for IV recombinant protein vs. 24-hours for ID vector administration). **C-D.** 8 mm thickness skin punch biopsies were taken at similar time points to measure local IL-12 and IL-2 concentrations. Cytokine concentrations were normalized to total protein. Data are displayed as means ± standard error of the mean (SEM) with symbols indicating independent animals (n=2-3 per group). Statistical significance was determined by a one-way ANOVA with Tukey's post-hoc analysis. **=p<0.01; ***=p<0.001; ****=p<0.0001.

Figure 3. Combinatorial KB703/KB704 via intratumoral injection enhances tumor regression and survival in B16F10 melanoma compared to control or single vector treatment



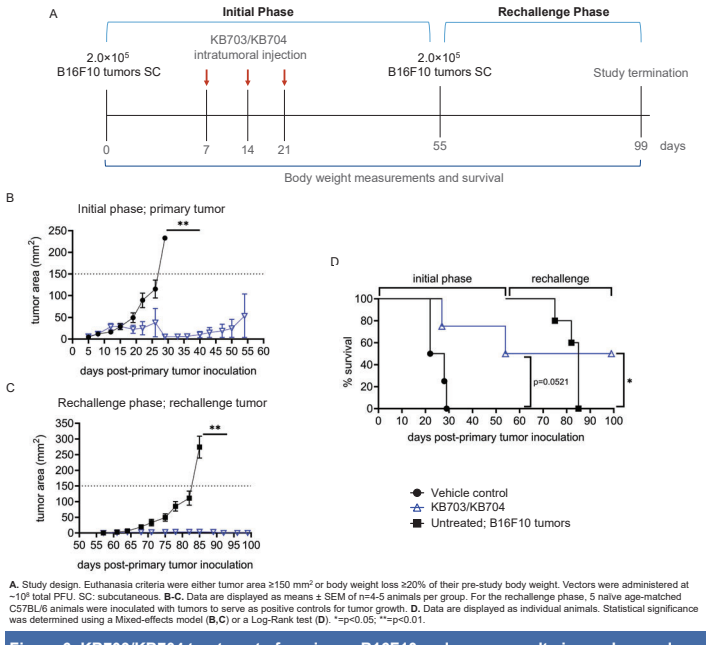
A. Study design. Euthanasia criteria were either tumor area ≥150 mm² or body weight loss ≥20% of their pre-study body weight. All vectors were administered at ~10^8 PFU. SC: subcutaneous **B.** Survival data are displayed as individual animals and were analyzed using a Log-Rank test corrected for multiple comparisons to compare each treatment group to vehicle control. ns=not significant; **=p<0.01.

Figure 4. Weekly KB703/KB704 intratumoral injection with additional maintenance dose improves survival of B16F10 melanoma-bearing mice



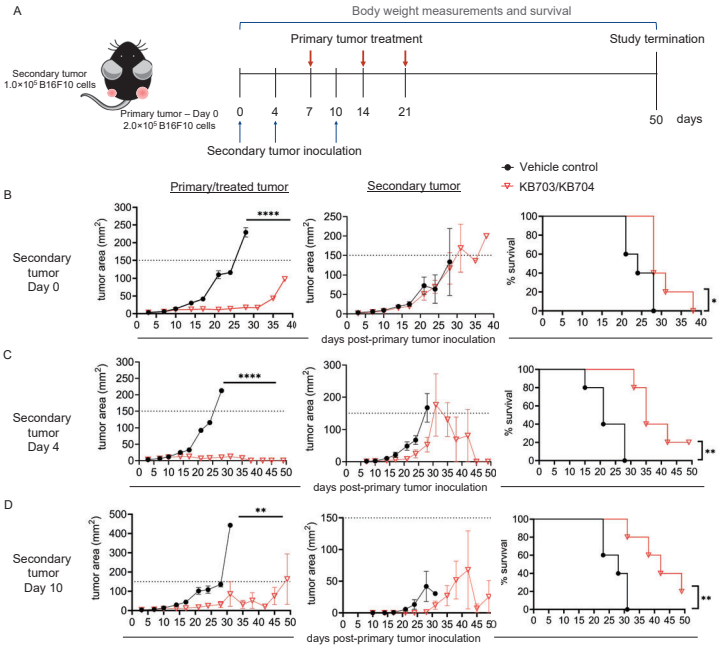
A. Study design. Euthanasia criteria were either tumor area ≥150 mm² or body weight loss ≥20% of their pre-study body weight. Vectors were administered at ~10^8 total PFU. SC: subcutaneous. **B.** Tumor measurements for each treatment group. Data are displayed as means ± SEM of n=10 animals per group and were analyzed using a Mixed-effects analysis. **C.** Survival data are displayed as individual animals and were analyzed using a Log-Rank test. ***=p<0.001.

Figure 5. KB703/KB704 treatment of primary B16F10 melanomas results in control of rechallenge tumors



A. Study design. Euthanasia criteria were either tumor area ≥150 mm² or body weight loss ≥20% of their pre-study body weight. Vectors were administered at ~10^8 total PFU. SC: subcutaneous. **B-C.** Data are displayed as means ± SEM of n=4-5 animals per group. For the rechallenge phase, 5 naïve age-matched C57BL/6 animals were inoculated with tumors to serve as positive controls for tumor growth. **D.** Data are displayed as individual animals. Statistical significance was determined using a Mixed-effects model (**B,C**) or a Log-Rank test (**D**). *=p<0.05; **=p<0.01.

Figure 6. KB703/KB704 treatment of a primary B16F10 melanoma results in an abscopal effect against a secondary B16F10 tumor



A. Schematic of tumor inoculation and study design. Euthanasia criteria were either tumor area ≥150 mm² or body weight loss ≥20% of their pre-study body weight. Vectors were administered at ~10^8 total PFU. **B-D.** Tumor measurements (left and center panels) are presented as means ± SEM of n=5 animals per group. Survival data (right panels) are displayed as individual animals. Statistical significance was determined using a Mixed-effects analysis (tumor area) or a Log-Rank test (survival). *=p<0.05; **=p<0.01; ****=p<0.0001

Conclusions

- Vector-driven expression of IL-12 and IL-2 minimized systemic cytokine exposure, while enhancing localized protein expression in the skin.
- Combinatorial therapy with IL-12 and IL-2 expressing vectors, KB703 and KB704, respectively, demonstrated a synergistic effect in the checkpoint inhibitor refractory B16F10 melanoma model, resulting in enhanced animal survival.
- KB703/KB704 treatment generated a durable anti-tumor memory response that was sufficient for recurrent tumor control.
- Treatment of primary tumors with KB703/KB704 resulted in at least partial secondary tumor growth inhibition and improved survival, suggesting an abscopal effect.

Acknowledgements/Disclosures/References

These studies were funded by Krystal Biotech, Inc.

Krystal Biotech, Inc. would like to thank Hilltop Lab Animals, Inc. for its 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.

1. Atkins MB, Robertson MJ, Gordon M, Lotze MT, DeCoste M, DuBois JS, et al. Phase I evaluation of intravenous recombinant human interleukin 12 in patients with advanced malignancies. Clin Can Res. 1997;3:409-417.
2. Rosenberg SA, Lotze MT, Muul LM, Leitman S, Chang AE, Ettinghausen SE, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. NEJM. 1985;313:1485-1492.
3. Food and Drug Administration. Guidance for industry estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers [Internet]. Rockville, MD: Center for Drug Evaluation and Research; Jul 2005. Available from: https://www.fda.gov/media/72309/download