Localized airway delivery of Interleukins-12 and -2 via inhalation of a replication-defective HSV-1-based therapy for the treatment of primary and secondary lung tumors

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Krystal

SITC 2023
Annual Meeting
POSTER #1066

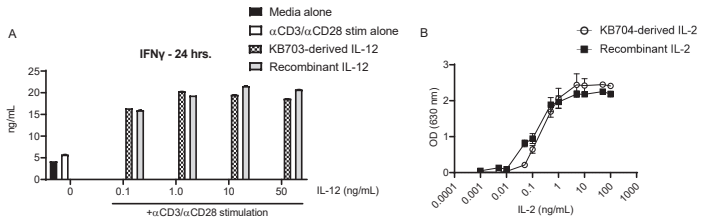
Abstract

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

Methods: For preclinical development, vectors were constructed to express murine *Il12* and *Il2*, termed KB703 and KB704, respectively, as the human cytokines are only partially cross-reactive in mice. Transgene bioactivity was confirmed *in vitro* prior to animal studies. Efficacy of localized IL-12 and -2 delivery was then tested in the K7M2 Osteosarcoma lung metastasis model. BALB/c mice were inoculated with K7M2 tumors intravenously (IV) on day 0, and cohorts were treated via inhalation with vehicle, single, or combinatorial vector therapy on days 14, 21, and 28.

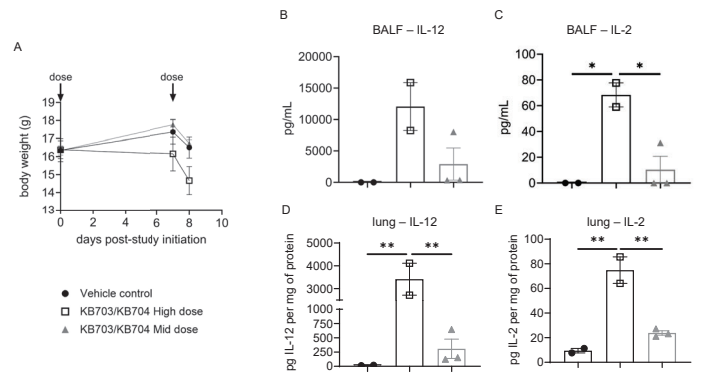
Results: *In vivo*, once weekly inhalation of combined KB703/KB704 was well tolerated in healthy mice, and IL-12 and IL-2 were detected in lung tissue and bronchoalveolar lavage fluid. Systemic cytokine exposure was also compared between inhaled vector recipients and animals treated IV with recombinant proteins at clinically relevant doses. Peak vector-derived IL-12 and IL-2 serum concentrations were considerably reduced compared to IV recombinant protein therapy, suggesting limited systemic cytokine exposure. In K7M2 studies, single agents demonstrated some efficacy over vehicle alone, yet combined KB703/KB704 therapy resulted in 100% overall survival at day 100 ($p<0.01$ compared to vehicle control). Histological analysis of lungs from KB703/KB704-treated animals revealed no tumor burden, with moderate peri-vascular and peri-bronchial immune cell infiltrate, suggesting immune-mediated control of tumor growth. A rechallenge study was then conducted where surviving KB703/KB704 dosed animals were reinoculated with K7M2 tumors IV 75 days after their first inoculation. Untreated, tumor recipient animals demonstrated a median survival of 36 days, while vector treated, rechallenged animals had a median survival >66 days post-rechallenge, suggesting a durable anti-tumor memory response.

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



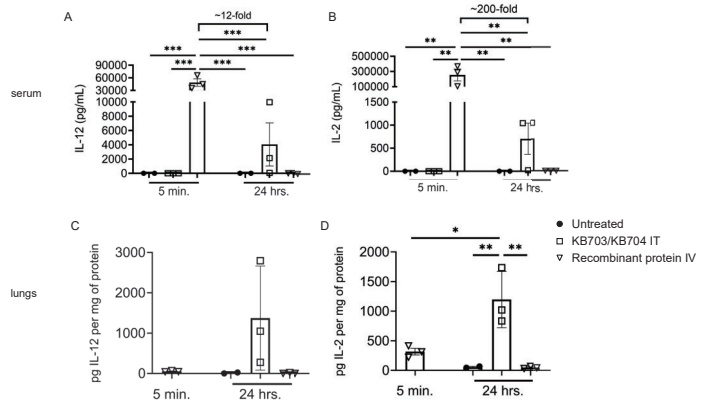
HEK 293FT cells were transduced with either KB703 (mIL-12) or KB704 (mIL-2) for 24-hours at a multiplicity of infection of 1. Supernatants were collected and cytokine concentrations were determined by ELISA (R&D systems). These supernatants served as the respective sources of vector-derived cytokines. **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. Repeated KB703/KB704 administered intratracheally is well-tolerated and results in detectable IL-12 and IL-2 expression in the lungs



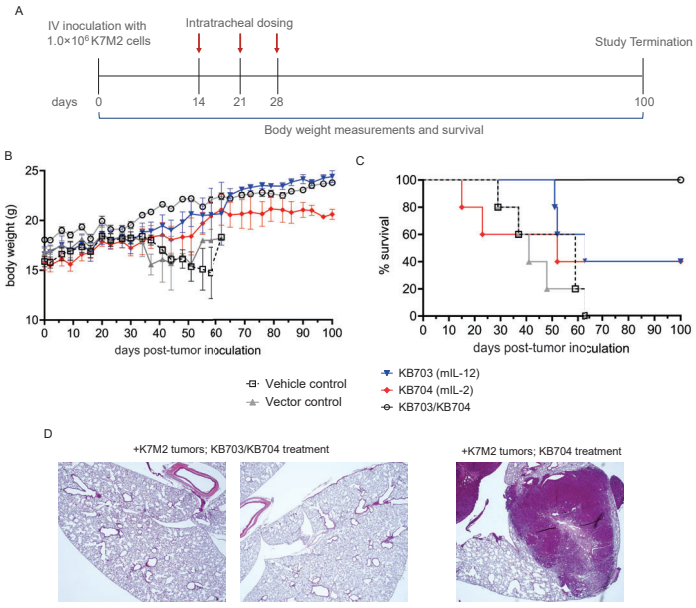
Healthy, BALB/c females were treated intratracheally (IT) with either vehicle control or KB703/KB704 high or mid dose (~10^6 or ~ 10^7 total PFU, respectively) on days 0 and 7. Animals were sacrificed on day 8, 24-hours following their last dose. **A.** Body weights were taken once weekly to assess toxicity. Data are displayed as means ± standard error of the mean (SEM). **B-E.** Bronchoalveolar lavage fluid (BALF) and whole lungs were collected postmortem and analyzed for IL-12 and IL-2 concentrations by ELISA (R&D Systems). **B-C.** BALF cytokine concentrations. **D-E.** Whole lung homogenate cytokine concentrations normalized to total protein. Data are displayed as means ± standard error of the mean (SEM), with symbols representing independent animals. n=2-3 per group. (**B-E**). Statistical significance was determined using a one-way ANOVA with Tukey's post-hoc analysis. *=p<0.05; **=p<0.01.

Figure 3. Intratracheal administration of KB703/KB704 minimizes systemic cytokine exposure while augmenting IL-12 and IL-2 concentrations in the lungs compared to recombinant proteins administered intravenously at clinically-relevant doses



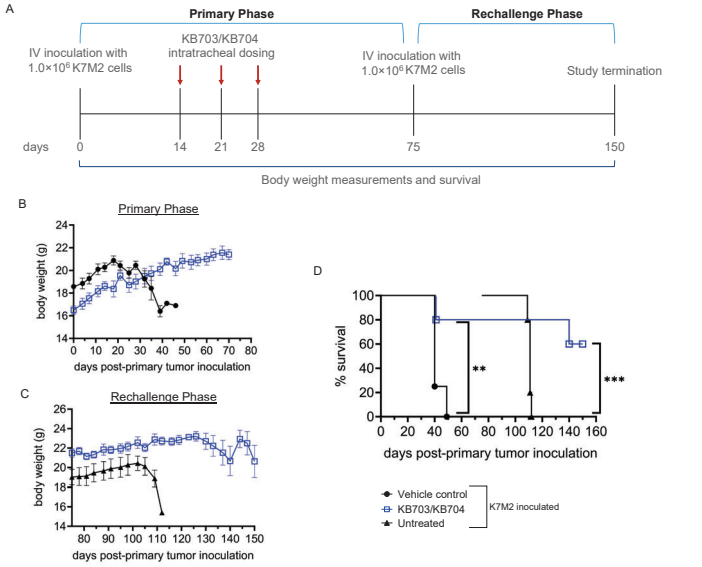
BALB/c mice were treated with KB703/KB704 (~10^7 total PFU) IT or IL-12 and IL-2 recombinant proteins (125 ng and 600 ng, respectively) IV at murine equivalent doses to human IL-12 and IL-2 that demonstrated clinical efficacy and toxicity in humans[1-3]. Untreated animals served as negative controls. **A-B.** Serum was sampled at 5 minutes and 24-hours post-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 IT vector administration). **C-D.** Whole lungs were taken at indicated time points to measure IL-12 and IL-2 concentrations. Cytokine concentrations were normalized to total protein. Data are displayed as means ± 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<0.05; **=p<0.01; ***=p<0.001.

Figure 4. Combinatorial KB703/KB704 administered intratracheally enhances tumor regression and survival in the K7M2 Osteosarcoma lung metastasis model compared to control or single vector treatment



A. Study design. Euthanasia was performed if body weight loss was ≥20% of their pre-study body weight. All vectors were administered at ~10^7 total PFU. IV – intravenous. **B.** Body weight data are displayed as means ± SEM with n=5 animals per group **C.** Survival data are displayed as individual animals. **D.** Representative H&E-stained lung sections from animals that survived to day 100. Statistical significance was determined using a Log-Rank test correcting for multiple comparisons (**C**). **=p<0.01.

Figure 5. Intratracheal administration of KB703/KB704 minimizes initial K7M2 lung tumor outgrowth and delays tumor recurrence without additional therapeutic intervention



A. Schematic of study design. Euthanasia was performed if body weight loss was ≥20% of their pre-study body weight. Vectors were administered at ~10^7 total PFU. For the rechallenge phase, 5 untreated age-matched BALB/c animals were inoculated with tumors to serve as positive controls for tumor growth. IV – intravenous. **B-C.** Body weight measurements of animals during primary (**B**) and rechallenge (**C**) phases. Data are displayed as means ± SEM of n=4-5 animals per group. **D.** Survival data are displayed as individual animals. Statistical significance was determined using a Log-Rank test. **=p<0.01; ***=p<0.001

Conclusions

- Vector-driven expression of IL-12 and IL-2 minimized systemic cytokine exposure while enhancing localized cytokine expression in the lungs.
- Combinatorial therapy of IL-12- and IL-2-expressing vectors, KB703 and KB704, respectively, demonstrated a synergistic effect in the K7M2 Osteosarcoma lung metastasis model, resulting in enhanced animal survival.
- KB703/KB704 treatment generated a durable anti-tumor memory response delaying tumor recurrence in a rechallenge model.

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.

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