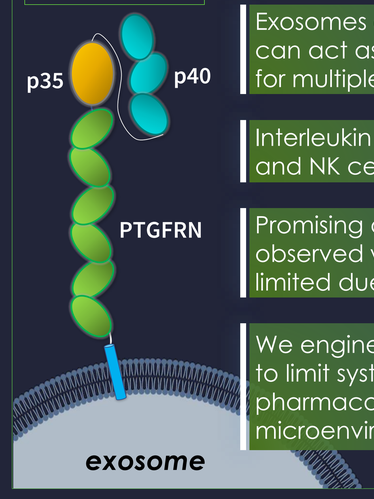
Exosome Surface Display of IL-12 Results in Tumor-Retained Pharmacology with Superior Potency and Limited Systemic Exposure Compared to Recombinant IL-12

Katherine Kirwin, Chang Ling Sia, Sonya Haupt, Ke Xu, Kevin Dooley, Su Chul Jang, Bryan Choi, Adam Boutin, Christine McCoy, Scott Estes, Kyriakos Economides, Sriram Sathyanarayanan, Nuruddeen Lewis

Codiak BioSciences, Cambridge, MA

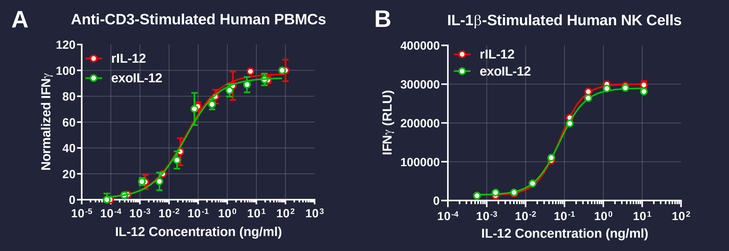
Introduction

exoIL-12™



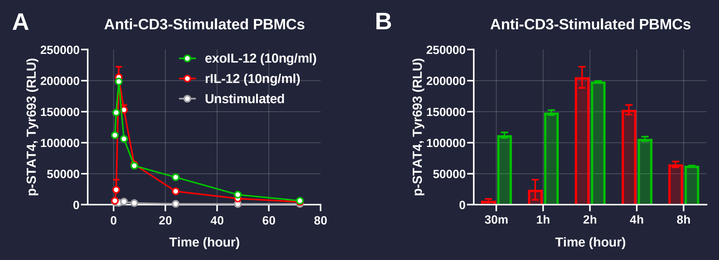
- Exosomes are natural, cell-derived vesicles that can act as a non-immunogenic delivery system for multiple therapeutic payloads
- Interleukin 12 (IL-12) promotes Th1 immunity, T cell and NK cell proliferation, and IFNγ production
- Promising anti-tumor responses have been observed with IL-12, but its clinical use has been limited due to toxicity
- We engineered IL-12 onto the exosome surface to limit systemic exposure and maximize local pharmacology within the tumor microenvironment

In Vitro Potency of exoIL-12 is Equivalent to Recombinant IL-12



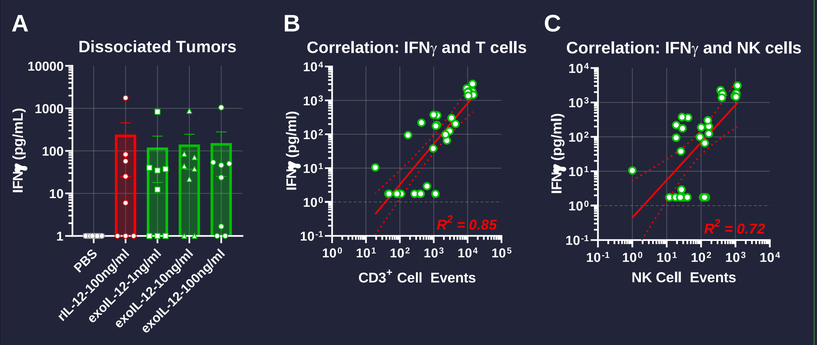
A. Human PBMCs were stimulated with anti-CD3 (1 ng/ml) and rIL-12 or exoIL-12 at various doses. IFNγ levels in the supernatant were assessed on day 4 by AlphaLISA. **B.** Human NK cells were isolated from whole blood using RosetteSep. Cells were stimulated with IL-1β (10 ng/ml) and rIL-12 or exoIL-12 at various doses. IFNγ levels in the supernatant were assessed on day 3 by AlphaLISA.

exoIL-12 Activates T cells Faster Than rIL-12



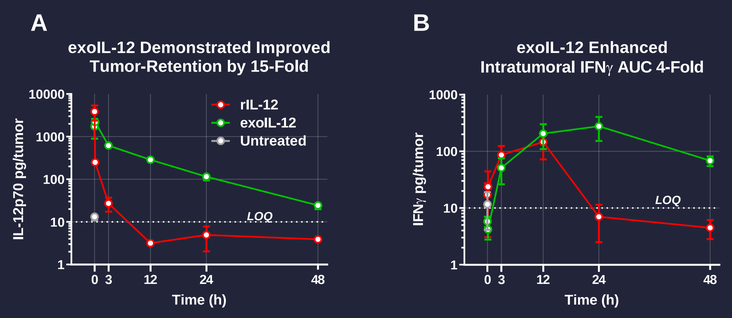
A-B. Human PBMCs were stimulated with anti-CD3 (2 ng/ml) for 3 days to induce IL-12 receptor expression. Cells were washed, counted, and re-plated. Cells were stimulated with rIL-12 or exoIL-12 (10 ng/ml), and lysates were prepared at various timepoints. Phospho-STAT4 levels were measured using the AlphaLISA SureFire Ultra kit.

exoIL-12 induces IFNγ in Dissociated Human Tumors



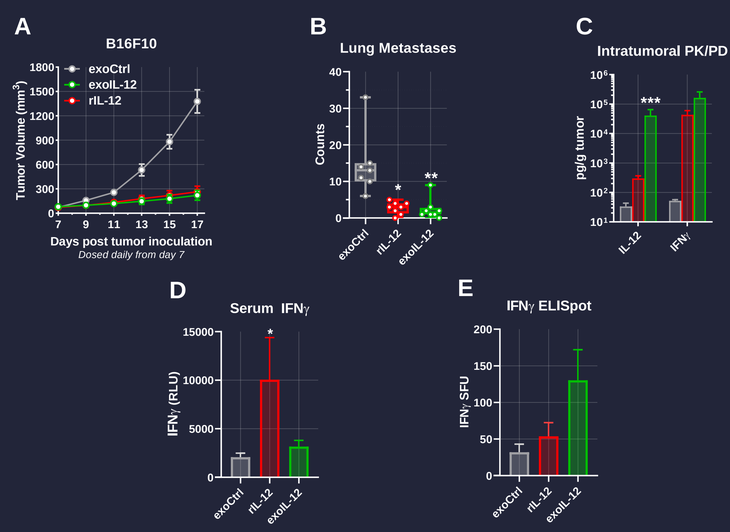
A-C. Dissociated human tumor biopsies were acquired from Discovery Life Sciences spanning five different tumor types. Cells were stimulated with rIL-12 or exoIL-12 at the indicated doses. Flow cytometry and cytokine analysis by LegendPlex was performed on day 5. In A, each dot represents a different donor. In B-C, all samples except the PBS group were included.

Enhanced PK and Sustained PD with Intratumoral Dosing of exoIL-12



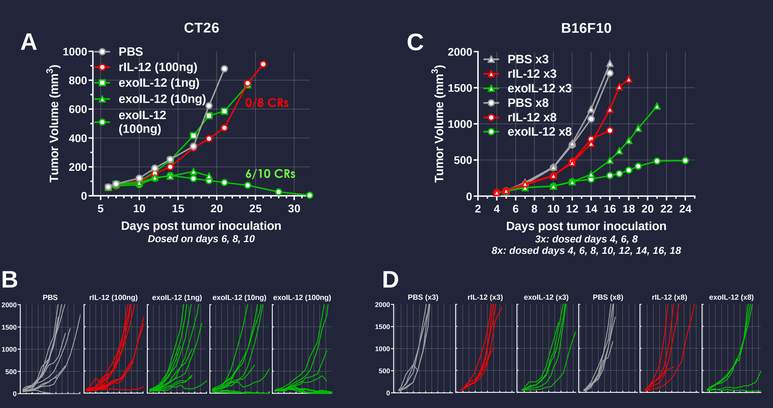
A-B. C57BL/6 mice were implanted subcutaneously with B16F10 cells on the flank. Recombinant IL-12 or exoIL-12 (100 ng) was injected intratumorally. Tumors were isolated and protein lysates were prepared and assessed for protein levels of IL-12 and IFNγ after 5 minutes, 3h, 12h, 24h, and 48h (n = 3). Time zero data was prepared by injecting rIL-12 or exoIL-12 into B16F10 tumors ex vivo and immediately processed. Limit of quantitation (LOQ).

Prolonged Intratumoral PK Provides Enhanced Systemic Immunity with Lower Levels of Systemic IFNγ



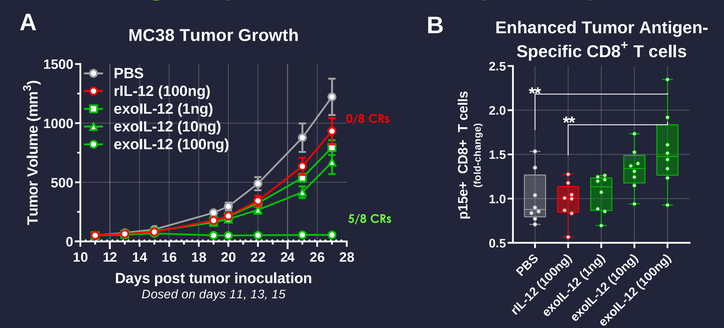
A. Recombinant IL-12 and exoIL-12 (100 ng) were tested in the B16F10 tumor model (n = 8). **B.** On day 5 post-inoculation, mice received an IV injection B16F10 cells. **C.** Intratumoral IL-12, IFNγ and (**D**) serum IFNγ levels were assessed at the end of the study. **E.** IFNγ ELISpot was performed using splenocytes stimulated with TRP2 and GP100 peptides.

Effective Tumor Growth Inhibition by exoIL-12 in Multiple Syngeneic Tumor Models



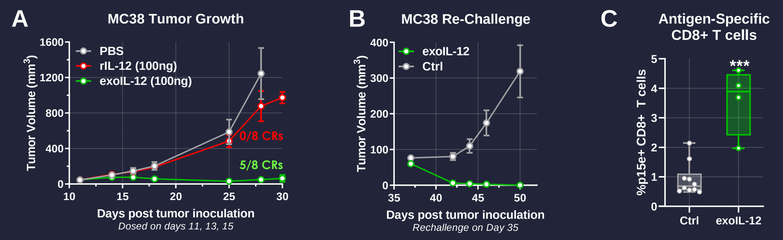
A-B. CT26 tumor cells were implanted subcutaneously in the flanks of mice (*n* = 10 per group). Recombinant IL-12 or exoIL-12 was dosed intratumorally at the indicated dose and frequencies. Geometric means are plotted in A. **C-D.** B16F10 tumor cells were implanted subcutaneously in the flanks of mice (*n* = 5 per group). Recombinant IL-12 or exoIL-12 was dosed intratumorally at 100 ng at the indicated frequencies. Geometric means are plotted in C.

Dose-Dependent Tumor Growth Inhibition and Systemic Tumor Antigen-Specific Immune Response by exoIL-12



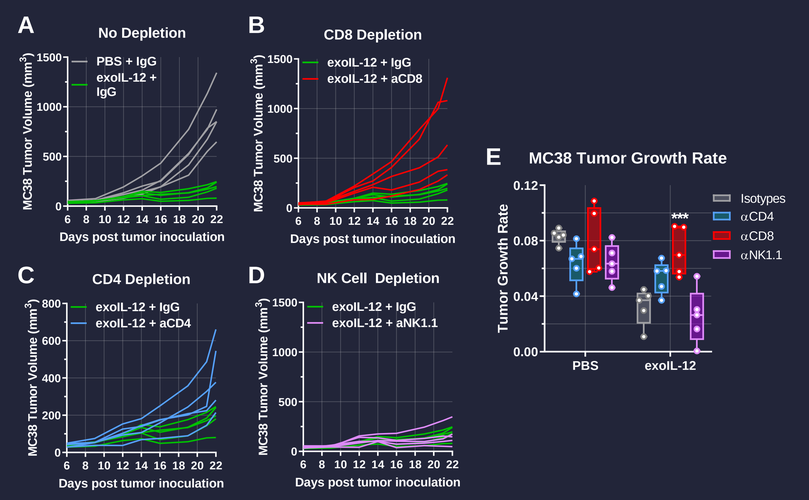
A. MC38 tumor cells were implanted subcutaneously into the flank and mice were dosed with rIL-12 or exoIL-12 at the indicated doses and indicated days (n = 8). Mean ± SEM is shown. **B.** Antigen-specific CD8+ T cells in the spleen were assessed by flow cytometry using tetramers for p15e on day 27.

Durable Complete Responses Observed with exoIL-12



A. Recombinant IL-12 and exoIL-12 (100 ng) were assessed for efficacy in the MC38 tumor model (n = 8). **B.** On day 35, mice that were complete responders were re-challenged with an additional MC38 inoculation. **C.** Antigen-specific CD8+ T cells were assessed in the spleen using p15e tetramers.

exoIL-12 Efficacy is Dependent on CD8+ T Cells



A-E. PBS **or** exoIL-12 was dosed intratumorally in the MC38 tumor model IT on days 9, 11, and 13. Antibodies were used to deplete specific immune cell types and were dosed intraperitoneally twice weekly starting on day 7. A: Isotype controls; B: anti-CD8 for CD8+ T cells; C: anti-CD4 for CD4+ T cells; D: anti-NK1.1 for NK cells. **E.** Tumor growth rates are shown for each group (n = 5).

Summary

- The enhanced PK/PD profile of exoIL-12 led to >100-fold improvement in tumor growth inhibition and superior induction of systemic tumor antigen-specific T cell responses
- In both MC38 and CT26 models, exoIL-12 treatment resulted in 60% complete responses, whereas equivalent amounts of rIL-12 produced no complete responses
- exoIL-12 may address the limitations of rIL-12 by increasing tumor retention, improving local IFNγ production, enhancing therapeutic anti-tumor activity, and reducing systemic release of cytokines
- Clinical development for exoIL-12 is planned to begin in the second half of 2020

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