Exenokine-2: a half-life extended no-α-IL-2 with improved preclinical pharmacological properties supports first-in-human clinical development

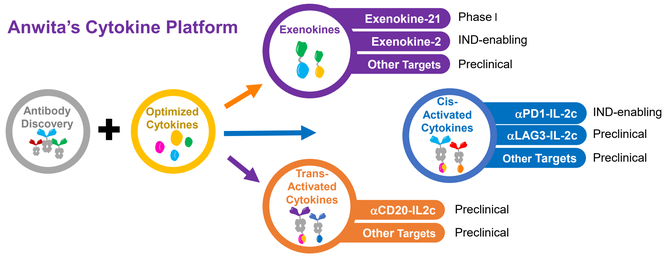
Xiaoli Cheng, Fan Ye, Sandra Chen, Jianing Huang, Jenny Jiang, Henry He, Shirley Shi,
Mingxing Yang, Qiang Fu, Ark Xia, Yudan Hong, Wang Xin, Karen Yuan, Hongchuan Liu*, Hui Feng*, Eugene Liu, and Ziyang Zhong

Lili Cheng lcheng@anwitabio.com
Ziyang Zhong zzhong@anwitabio.com
300 Industrial Rd. San Carlos, CA 94070

Abstract Number: 1081

INTRODUCTION

IL-2 is a critical cytokine driving immune mediated killing of tumor cells by stimulating both the innate and adaptive immune cells. High-dose IL-2 (aldesleukin) has been approved for the treatment of metastatic melanoma and metastatic renal cell carcinoma[1], however, the practical use of aldesleukin in the clinic is limited. The short half-life of aldesleukin necessitates a frequent and burdensome treatment schedule for patients. In addition, binding to IL-2Rα on endothelial cells and type 2 innate lymphoid cells is thought to induce severe adverse events associated with vascular leak syndrome (VLS)[2]. Furthermore, the efficacy of aldesleukin is compromised due to strong activation of immunosuppressive regulatory T cells (Treg) expressing the high-affinity IL-2Rαβγ[3]. To overcome the major limitations of wild-type (WT) IL-2, Exenokine-2 (Exn-2), a fusion protein comprised of a no-α-IL-2 linked to a humanized anti-human serum albumin single domain antibody, was designed using Anwita's proprietary discovery technology.



Exn-2 Is a No-α, βγ-Selective Binder of IL-2R

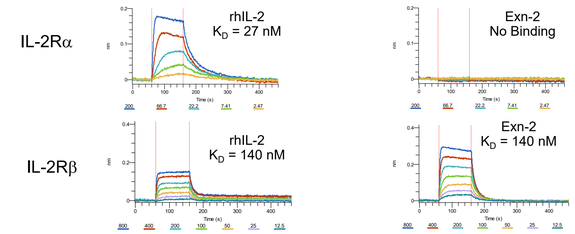


Figure 1. Exn-2 does not bind to IL-2Rα and binds to IL-2Rβ with similar affinity as rhIL-2. Binding affinities to IL-2 receptors were determined by Bio-Layer Interferometry

Exn-2 Exhibits Reduced Bias Towards Treg

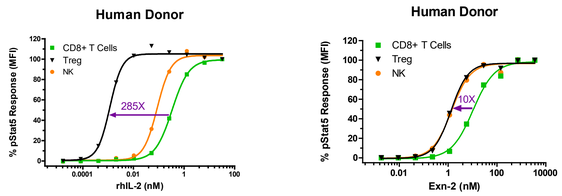
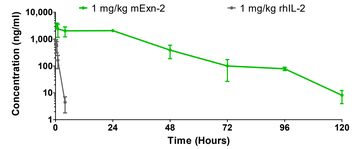


Figure 2. Potency towards immunosuppressive Tregs is significantly reduced in Exn-2. STAT5 phosphorylation in primary human CD8+ T cells, NK cells and Tregs after stimulation with rhIL-2 (Left) or Exn-2 (Right). Data is representative from multiple human donors.

mExn-2 Exhibits Improved Systemic Exposure in Mice



PK Parameter	mExn-2	rhIL-2
$t_{1/2}$ (h)	15.8	0.5
T_{max} (h)	0.5	0.3
C_{max} (ng/mL)	3,280	813
$AUC_{0\text{-}inf}$ (ng/mL*h)	90,236	1,002

Figure 3. Half-life of mExn-2 is extended in mice as compared to rhIL-2. Plasma concentrations of mExn-2 and rhIL-2 in mice following a single IP dose. (Average ± SEM of three mice)

mExn-2 Exhibits Robust Single Agent Anti-tumor Activity

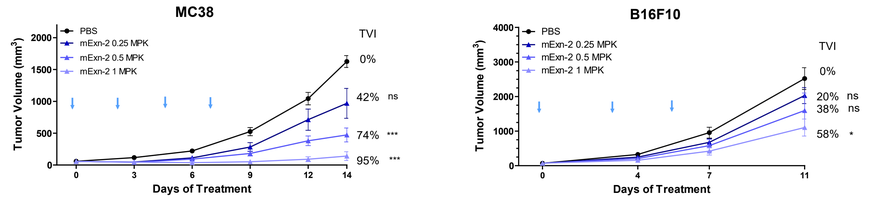


Figure 4. Single agent anti-tumor efficacy from mExn-2 is robust and dose-dependent in mouse syngeneic models. MC38 (Left) or B16F10 (Right) tumor-bearing mice were treated with monotherapy mExn-2 injected IP BIW at 0.25, 0.5 and 1 mg/kg. (Average ± SEM of 6 mice per group)

mExn-2 Enhances Anti-tumor Activity of Anti-mPD1

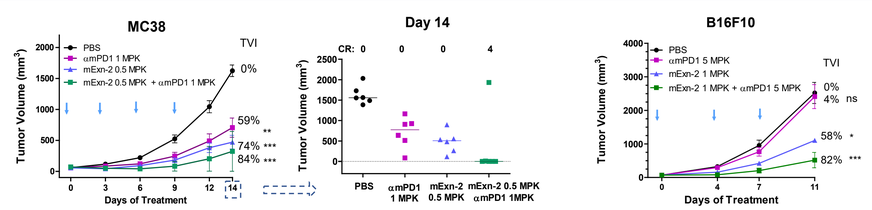


Figure 5. mExn-2 synergizes with anti-mPD1 resulting in 66% complete response (CR) rate in MC38 tumor-bearing mice. MC38 (Left) or B16F10 (Right) tumor-bearing mice were treated with mExn-2 (0.5 mg/kg), anti-mPD1 (1 mg/kg), or with a combination of mExn-2 and anti-mPD1 injected IP BIW. (Average ± SEM of 6 mice per group)

mExn-2 Enhances Anti-tumor Activity of TRAS and RTX

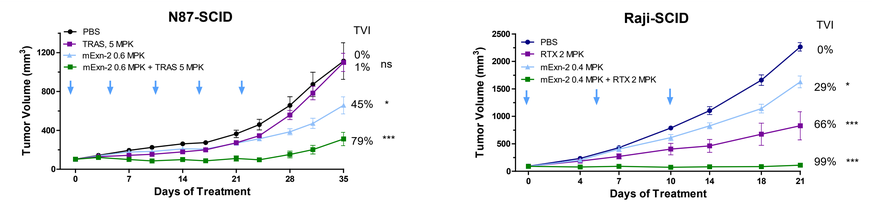
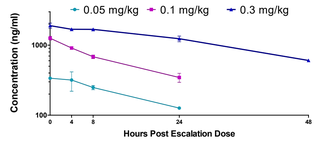


Figure 6. mExn-2 synergizes with ADCC-competent Trastuzumab (TRAS). N87 tumor-bearing SCID mice were treated with mExn-2, TRAS, or with a combination of mExn-2 and TRAS injected IP QW. (Average ± SEM of 8 mice per group)

Figure 7. mExn-2 synergizes with ADCC-competent Rituximab (RTX). Raji tumor-bearing SCID mice were treated with mExn-2, RTX, or with a combination of mExn-2 and RTX injected IP QW. (Average ± SEM of 8 mice per group)

Exn-2 Exhibits Improved Systemic Exposure in NHP



PK Parameter	0.05 mg/kg	0.1 mg/kg	0.3 mg/kg
$T_{1/2}$ (h)	15	15	29
C_{max} (ng/mL)	372	1,258	1,900
$AUC_{0\text{-}inf}$ (ng/ml*h)	8,152	23,187	84,670

Figure 8. Half-life of Exn-2 is extended in cynomolgus monkeys. Serum concentrations of Exn-2 in cynomolgus monkeys following IV doses of 0.05 mg/kg, 0.1 mg/kg, and 0.3 mg/kg every 14 days.

Exn-2 Induces Expansion of Lymphocyte and CD8+ in NHP

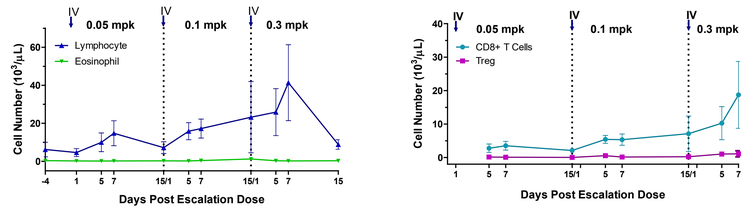


Figure 9. Exn-2 selectively expands lymphocytes and CD8+ T cells with no or minimal impact on eosinophils and Tregs in cynomolgus monkeys. Counts of lymphocyte and eosinophile (Left), or CD8+ T cells and Tregs (Right) from cynomolgus monkeys following IV doses of Exn-2 at 0.05 mg/kg, 0.1 mg/kg, and 0.3 mg/kg every 14 days.

CONCLUSIONS

- Exn-2 is a no-α, βγ-selective agonist of IL-2R exhibiting significantly reduced bias towards Treg than WT IL-2.
- Exn-2 demonstrates robust dose-dependent single-agent activity and enhances anti-tumor activity when combined with anti-PD1, trastuzumab or rituximab.
- In NHP, Exn-2 induces pronounced and sustained expansion of lymphocytes and CD8+ T cells with no or minimal effects on eosinophils and Treg cells.
- Exn-2 is well tolerated and no major clinical findings or signs of VLS have been observed.
- GMP manufacturing has been completed producing sufficient drug product to support Phase1 clinical study.

REFERENCES

1. Payne, R. et al. Durable responses and reversible toxicity of high-dose interleukin-2 treatment of melanoma and renal cancer in a community hospital biotherapy program. *J. Immunother. Cancer* **2**, 13 (2014).
2. Waldmann, T. A. Cytokines in cancer immunotherapy. *Cold Spring Harb. Perspect. Biol.* **10**, 12 (2018).
3. Krieg C et al. Improved IL-2 immunotherapy by selective stimulation of IL-2 receptors on lymphocytes and endothelial cells. Proc Natl Acad Sci **107**, 26 (2010).

ABOUT ANWITA BIOSCIENCES

Anwita Biosciences, Inc. is a privately held emerging biopharmaceutical company headquartered in the San Francisco Bay Area. Anwita specializes in the discovery and development of optimized immunotherapies for the treatment of cancer and autoimmune diseases, leveraging on core expertise in cancer immunotherapy, bioinformatics, and structure-based protein engineering. Our pipeline expanses from discovery to Phase 1 and encompasses half-life extended cytokines, antibody-cytokine conjugates (see STIC2022 Abstracts 1104 and 1188) and other formats.

* Junshi Biosciences, 15F, Building 7, No.6, Lane 100, Pingjiaqiao Road, Pudong New Area, Shanghai, China