AWT030: A First-in-class Bi-functional IL-21 Fusion Protein Selectively Activates Tumor Infiltrated CD8 T Cells and Suppresses Treg Cells

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Introduction

Immune suppressive tumor microenvironment (TME) has been identified as a significant obstacle to the success of immunotherapy, such as anti-PD-1 therapy. The TME comprises several factors that limit the response rate to immunotherapy, including immunosuppressive cells such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and M2 type macrophages, which directly or indirectly interact with effector cells and undermine their activation, proliferation, and survival, as well as their ability to recognize tumor cells[1-3].

AWT030 is a novel therapeutic approach designed to overcome the suppressive TME by selectively activating tumor-infiltrating CD8 T cells, suppressing Tregs, and avoiding the potential suppressive effect of IL-21 on dendritic cells. AWT030 is composed of a stability and potency optimized IL-21 mutein, an engineered functional domain that targets T cells, and an engineered Fc domain. In preclinical studies, AWT030 showed promising results as a monotherapy in multiple PD-1 insensitive tumor models and exhibited a synergistic effect with anti-PD-1 therapy. Immunophenotyping revealed that AWT030 had minimal impact on circulating lymphocytes while ameliorate the suppressive TME, with significantly increased CD8 T cell population and reduced Treg function. These results suggest that AWT030 has the potential to serve as a new modality of immunotherapy.

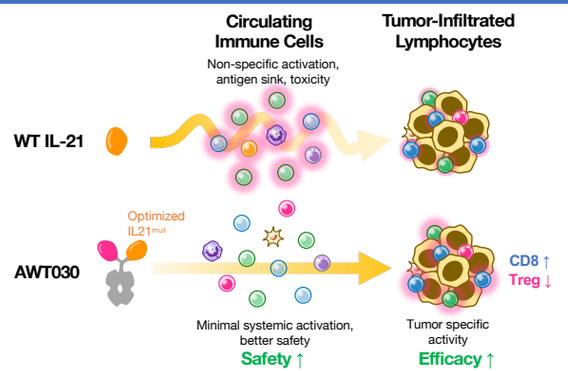
About Anwita Biosciences

Headquartered in the San Francisco Bay Area, Anwita Biosciences, Inc. is a privately held emerging biopharmaceutical company that focuses on the discovery and development of optimized cytokine fusion proteins for treatment of cancers. Leveraging its core expertise in cytokine biology, cancer immunotherapy, bioinformatics, and structure-based protein engineering, Anwita Biosciences has developed a pipeline encompasses half-life extended cytokines and tumor targeted cytokine fusions that are in different stages of development ranging from early discovery to Phase I.

Anwita's Tumor Targeting Cytokines

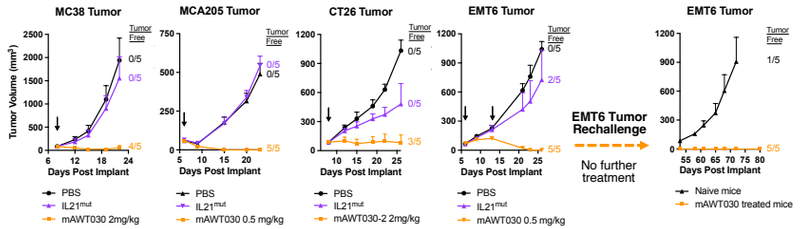
Program	MOA	Lead ID	Lead OP	IND Enabling	Phase 1
AWT020 (αPD1-IL2)	Tumoral CD8 ↑ ↑	Best-in-Class			
AWT026 (αLAG3-IL2)	Tumoral CD8 ↑ ↑	First-in-Class			
AWT030	Tumoral CD8↑ Treg↓	First-in-Class			

AWT030 is a Tumor Targeting Cytokine



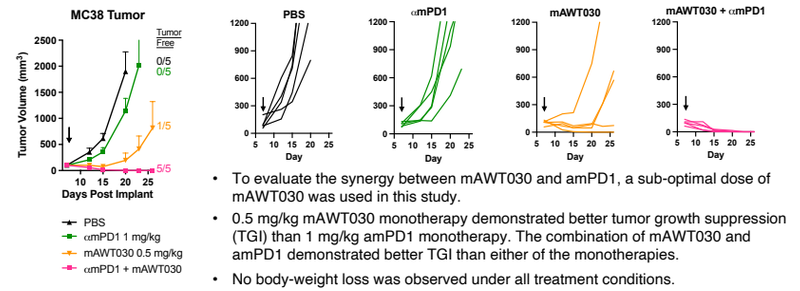
Superior Anti-Tumor Activity as Monotherapy

Tumor models that are less sensitive to PD1 blockade



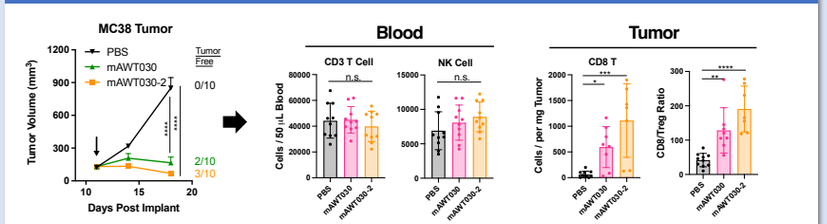
- The mouse surrogates of AWT030 (mAWT030) were tested in multiple tumor models, including tumor models that are less sensitive to anti-PD1 therapy.
- mAWT030 demonstrated excellent anti-tumor activity and induced long-lasting immunological memory.
- No body-weight loss was observed under all treatment conditions.

mAWT030 Synergies with Anti-PD1 Therapy



- To evaluate the synergy between mAWT030 and amPD1, a sub-optimal dose of mAWT030 was used in this study.
- 0.5 mg/kg mAWT030 monotherapy demonstrated better tumor growth suppression (TGI) than 1 mg/kg amPD1 monotherapy. The combination of mAWT030 and amPD1 demonstrated better TGI than either of the monotherapies.
- No body-weight loss was observed under all treatment conditions.

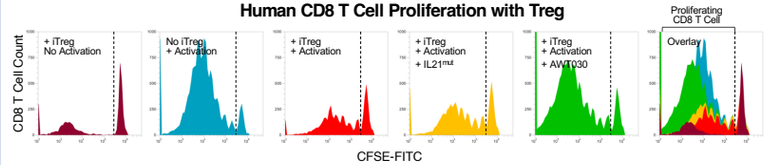
mAWT030 Selectively Targets TME



- 7 days after the first dose, blood and tumor samples were collected from each treatment group for immune cell phenotyping.
- In the blood, there was no significant change in circulating T cell and NK cell populations between the PBS and mAWT030-treated groups.
- In tumors, mice treated with mAWT030 had a significantly higher number of CD8 T cells and an increased CD8/Treg ratio.

AWT030 Protects CD8 T Cell From Treg Suppression

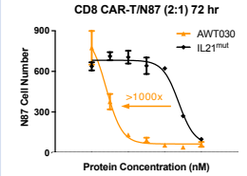
Human CD8 T Cell Proliferation with Treg



- Purified PBMC cells were labeled with CSFE and co-cultured with iTreg at 1:1 ratio
- After 6 days, CD8 T cell population was analyzed by Flow Cytometry.
- The AWT030-treated group had a much higher number of CD8 T cells.

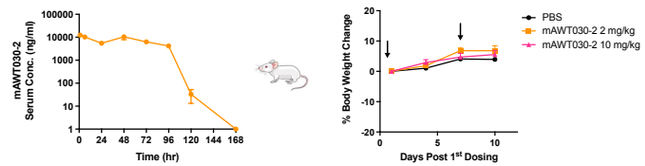
AWT030 Promotes Cytotoxicity of CD8 T Cell

CD8 CAR-T/N87 (2:1) 72 hr



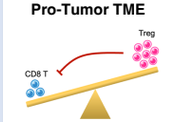
- An in-house developed anti-MSLN-CAR-T was used for the N87 cell killing assay
- CAR-T and N87 cells were mixed at a ratio of 2:1.
- The cell mixture was incubated at 37°C and 5% CO2 for 72 hours.
- Remaining live N87 cells were stained and counted by Cytation
- AWT030 significantly enhanced the cytotoxicity of CD8 CAR-T cells

mAWT030 Has Extended Half-life and Excellent Safety



- mAWT030-2 is a Fc fusion protein. Compared to rhIL21, the half-life of mAWT030 is significantly increased.
- mAWT030-2 is tolerated at 10 mg/kg in BALBc mice.

Conclusion



- Tumor growth
- Unresponsive to immunotherapy (e.g. αPD1)

- Tumor shrinkage
- Responsive to immunotherapy

mAWT030 is an innovative immunotherapy drug that selectively activates effector T cells and suppresses Treg function in the tumor microenvironment (TME). It has shown promising anti-tumor activity in multiple murine tumor models. In vitro assays support its mechanism of action by demonstrating enhanced CD8 T activity and reduced Treg suppression function. mAWT030 has the potential to become the next-generation immune therapy for cancers, offering a more effective and safe treatment option.

References

1. O'Donnell JS, et al., Nat Rev Clin Oncol. 2019
2. Beatty GL, et al., Clin Cancer Res. 2015
3. Hodi FS, et al. N Engl J Med. 2010

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