ATAK™ receptors: A new class of chimeric antigen receptors that harness innate immunity in myeloid cells to target cancer

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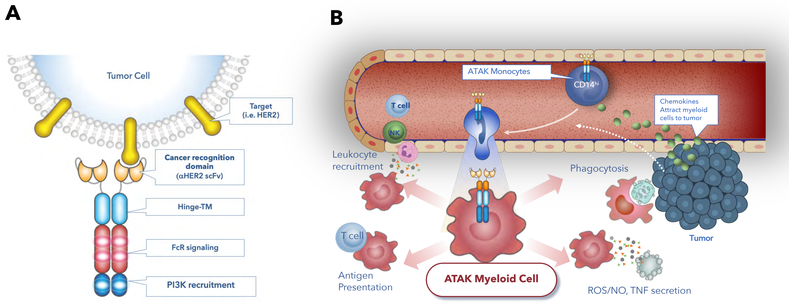
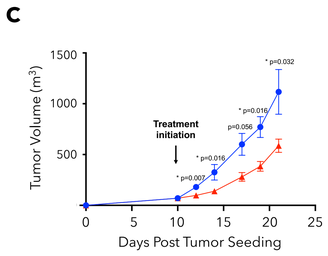
Abstract

For most patients with advanced solid tumors, sustained clinical benefit with immunotherapy has yet to be achieved. Myeloid cells, including monocytes and macrophages are the primary orchestrators of immune responses and are found to accumulate in tumors, in some cases contributing up to 75% of the tumor mass. Myeloid cells express a wide range of innate immune sensors such as Toll-like receptors, RIG-I and cGAS-STING as well as co-stimulatory molecules like CD40. The activation of these innate immune pathways in myeloid cells can be associated with anti-tumor immune response. Notwithstanding the ability of the myeloid cells to infiltrate tumors and elicit broad immune responses, technologies capable of harnessing these cells to target cancer remains elusive. Here we designed and engineered a new class of chimeric antigen receptors that couple tumor recognition with multiple innate immune signaling domains, referred to as **A**ctivate, **T**arget, **A**ttack & **K**ill (ATAK™) receptors. By combining tumor-associated antigen recognition domains with intracellular signaling domains from innate immune receptors such as FcR, TLR and cytokine receptors, we show that myeloid cells can be controlled and programmed to recognize cancer and elicit a broad and tunable immune response. In mice with established, highly immunosuppressive B16 melanoma tumors, delivery of monocytes engineered to express ATAK receptors results in anti-tumor activity. Our data show the versatility of building ATAK receptors by harnessing innate immune pathways and support their clinical development in cell therapies. A Phase I/II study to test the safety, tolerability and efficacy of 1st generation ATAK™-receptor in patients with T cell Lymphoma is currently on-going.

Introduction

The ATAK™ Myeloid Cell Platform





- Significant myeloid cell infiltration is observed in tumors, including T cell lymphoma (TCL), and solid tumors like ovarian, breast, gastric & esophageal cancer.
- The ability to engineer these cells represents a novel method to overcome the immunosuppressive conditions of the TME and promote immune rejection of tumors
- Our goal here is to further expand the potential of this approach by incorporating innate immune signaling domains to enhance the anti-tumor activity of ATAK receptors.

Figure 1: ATAK receptors program monocyte to kill tumor by mobilization of innate and adaptive immune system (A) Design of 1st Gen ATAK receptor based on Morrissey et al[1] (B) Mechanisms of action for ATAK myeloid cells. (C) 1st Gen ATAK receptor shows anti-tumor efficacy in T cell lymphoma animal model (H9 CTCL cells established as s.c. tumors in NSG mice)

Results

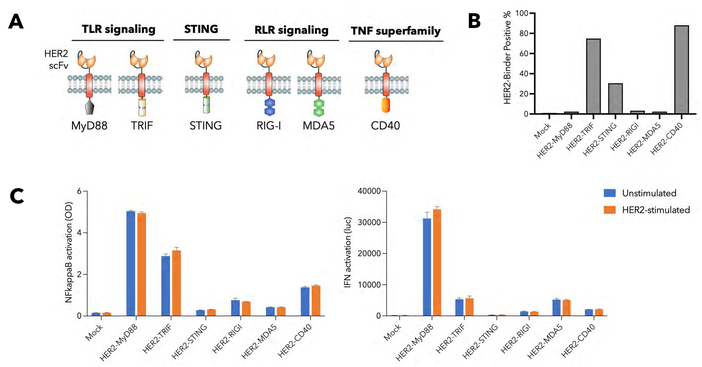


Figure 2: Design and screening of constructs with novel inflammatory signaling domains. (A) A broad array of inflammatory signaling domains are fused to a HER2 binding scFv domain and their pro-inflammatory signaling potential are examined. Domains are selected from inflammatory signaling receptors from TLR pathway, STING, RLR pathway and TNF superfamily of receptors. (B) Expression level of different ATAK receptors (C) ATAK receptors exhibit different levels of tonic signaling and activation of NFκB or Type-I Interferon (IFN) genes.

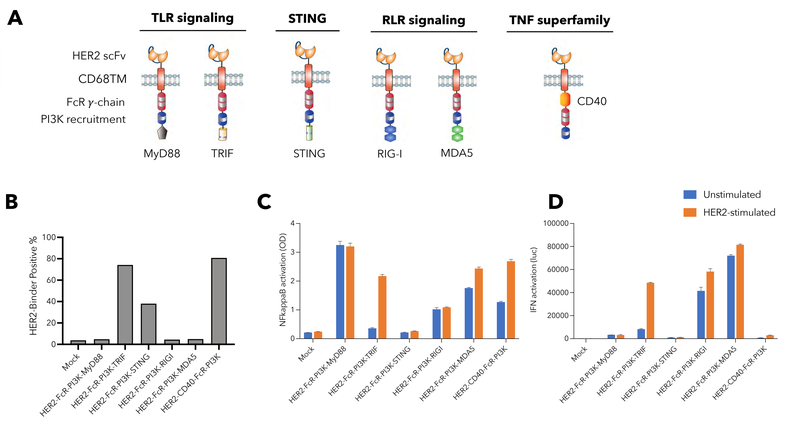


Figure 3: Design and screening of 2nd Gen ATAK constructs containing combined phagocytosis, PI3K & inflammatory signaling domains. (A) Diagrams of multi-domain ATAK receptors that contain HER2 scFv, CD68 transmembrane (TM) domain, FcR, PI3K and proinflammatory domains. (B) Expression levels of these tri-signaling domain ATAK receptors in THP1 cells. (C) and (D) Multi-domain ATAK constructs induce potent, NF-κB and IFN pathway activation in THP1-Dual cells following antigen-specific stimulation.

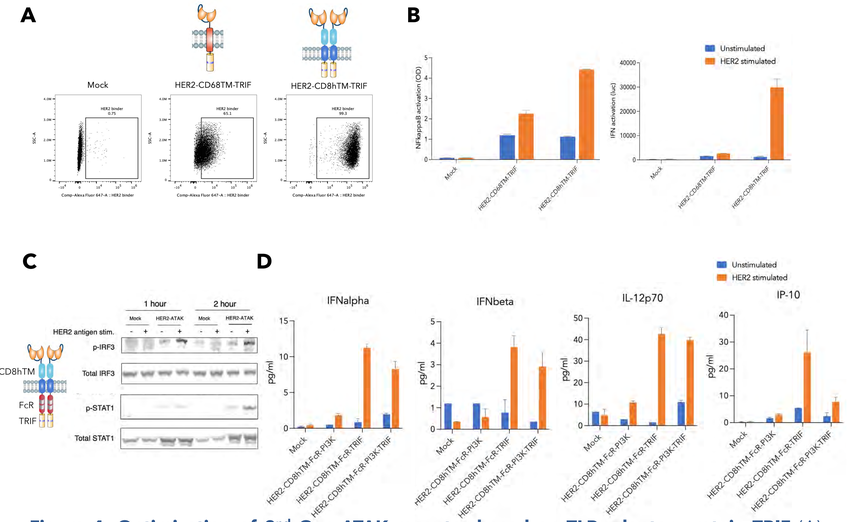


Figure 4: Optimization of 2nd Gen ATAK receptor based on TLR adaptor protein TRIF (A) Replacing the CD68 transmembrane domain with CD8 hinge-transmembrane domain significantly improves expression levels of TRIF based ATAK receptor. (B) CD8 hinge-TM improves antigen specific induction of NFκB and IFN-I signaling. (C) HER2 antigen stimulation of TRIF based 2nd Gen receptor promotes IFN-I signaling as indicated by increases of phosphorylation of IRF3 and STAT1. (D) HER2 antigen stimulation of TRIF based 2nd Gen receptor upregulates secretion of IFN-I pathway associated cytokines and chemokines that have anti-tumor activities.

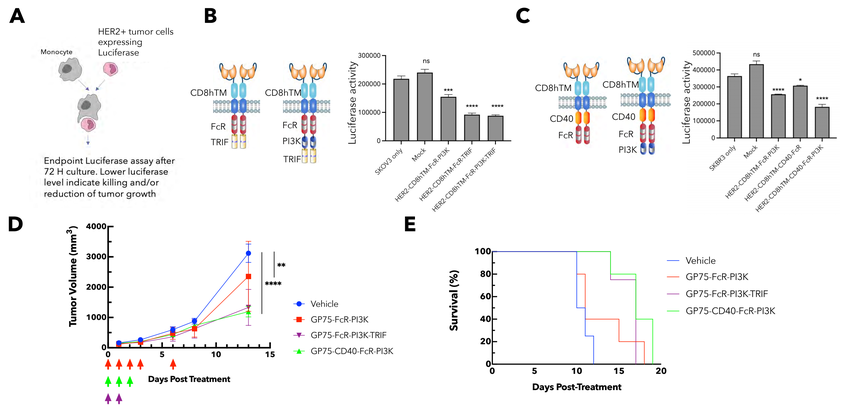


Figure 5: ATAK monocytes armed with 2nd Gen receptors exhibit anti-tumor activities in vitro and in vivo. (A) In vitro tumor killing assay to assess the ability of HER2-ATAK monocyte to kill HER2+ tumor cells. (B) Monocytes armed with TRIF based 2nd Gen ATAK receptor kill HER2+ tumor in vitro. (C) Monocytes armed with CD40 based 2nd Gen ATAK receptor kill HER2+ tumor in vitro. (D) 2nd Gen ATAK receptors with TRIF or CD40 show strong anti-tumor activity in B16 melanoma syngeneic model. (E) Survival of tumor-bearing mice treated with ATAK monocytes. Monocytes armed with 2nd Gen receptors show increased potency when compared to monocytes armed with 1st Gen receptors.

Conclusions

- We identified TRIF and CD40 signaling domain that can enhance the anti-tumor function of ATAK monocytes by promoting pro-inflammatory signaling pathways in response to tumor antigen.
- Human monocytes armed with TRIF or CD40 signaling domain ATAK receptors demonstrated antigen specific activation of NF-κB and IFN signaling pathways, culminating in tumor cell killing & inflammatory cytokine production.
- In a B16 melanoma syngeneic model, mouse monocytes armed with TRIF or CD40 ATAK receptors exhibited strong anti-tumor efficacy, resulting in delayed tumor progression and significantly prolonged survival.

References

1. Morrissey MA et al. Chimeric antigen receptors that trigger phagocytosis. Elife. 2018 Jun 4;7:e36688