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

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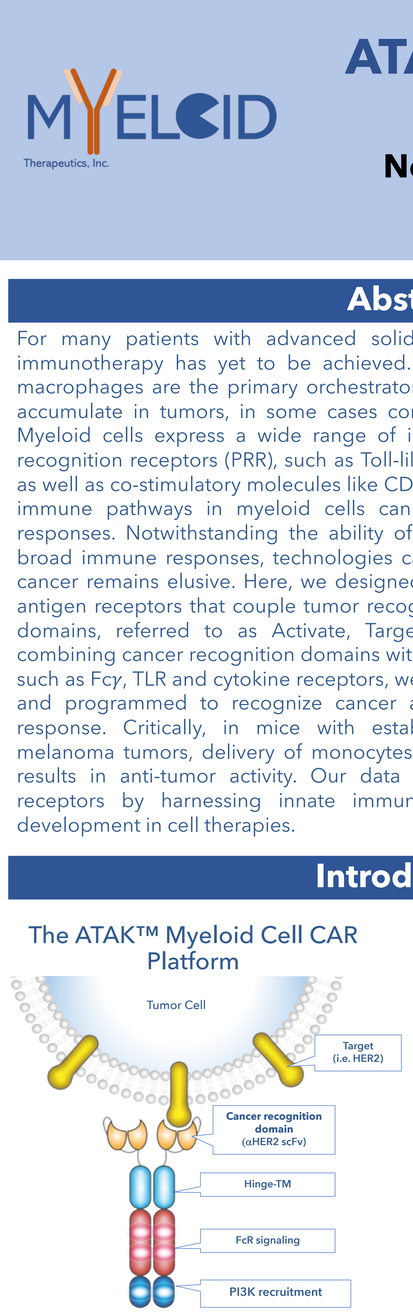
Abstract

For many 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, called as pattern recognition receptors (PRR), such as Toll-like receptors (TLRs), RIG-I and cGAS-STING as well as co-stimulatory molecules like CD40. The activation of these PRRs and innate immune pathways in myeloid cells can be associated with anti-tumor immune responses. Notwithstanding the ability of these 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 signal domains, referred to as Activate, Target, Attack & Kill (ATAK™) receptors. By combining cancer recognition domains with intracellular signaling domains from PRRs such as Fcγ, 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. Critically, 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.

Introduction

The ATAK™ Myeloid Cell CAR Platform



- Significant monocyte 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 solid tumors
- 1st generation ATAK™-receptors containing FcR and PI3K signaling domains are under clinical investigation in T cell lymphoma
- Our goal here is to further expand the potential of this approach by incorporating further immune signaling domains to further enhance the activity of 1st Gen ATAK™ receptors.

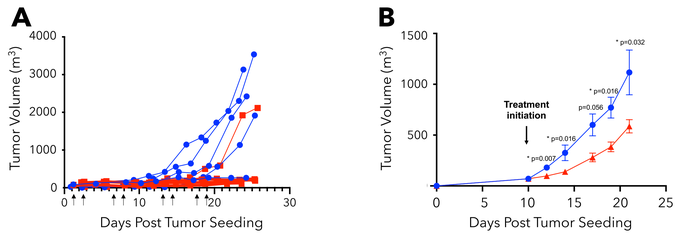


Figure 1: Murine monocytes engineered with first generation FcR-PI3K receptors show potent anti-tumor activity. Monocytes expressing GP75-FcR-PI3K show potent activity against (A) early and (B) established B16 tumors.

Results

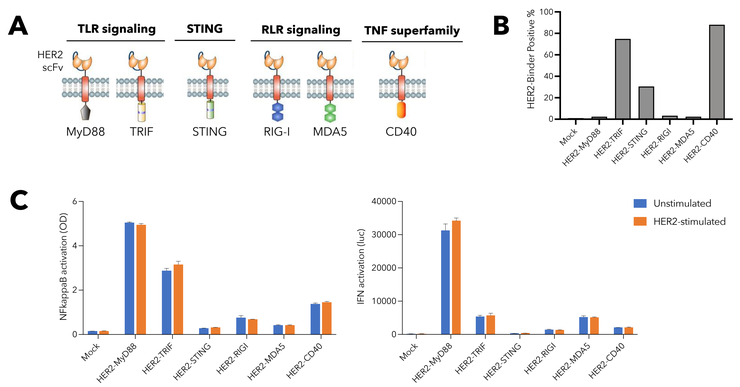


Figure 2: Design and screening of constructs with novel inflammatory signaling domains. A broad array of inflammatory signaling domains were fused to a HER2 binding scFv domain and their antigen specific signaling potential examined. Domains are selected from inflammatory signaling receptors from TLR pathway, STING, RLR pathway and TNF superfamily of receptors (A). In THP1 cells, receptors were expressed using mRNA and showed differential expression profiles (B). Interestingly, this data showed that these receptors can be expressed in THP1 cells, although differential impacts on signaling are observed, in some cases tonic NF-κB and IFN pathway activation was observed (Data are representative of experiments performed at least 3 times, with mean and St Dev).

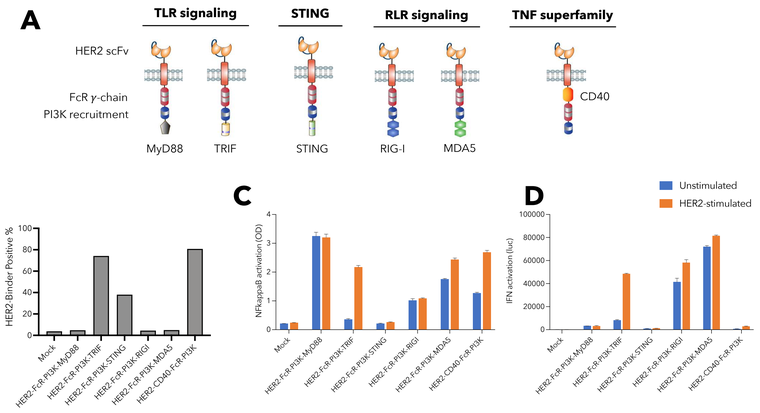


Figure 3: Design and screening of 2nd Gen ATAK constructs containing combined phagocytosis, PI3K & inflammatory signaling domains. (A) Rationale designed novel inflammatory signaling domains were further added to the baseline FcR-PI3K ATAK™-receptor resulting in multi-domain ATAK receptors. (B) In most cases, expression of these tri-signaling domain receptors was observed in THP1 cells. Importantly, multi-domain ATAK constructs induce potent, NF-κB and IFN pathway activation in THP1-Dual cells following antigen-specific stimulation. (C&D, Data are representative of experiments performed at least 3 times, with mean and St Dev).

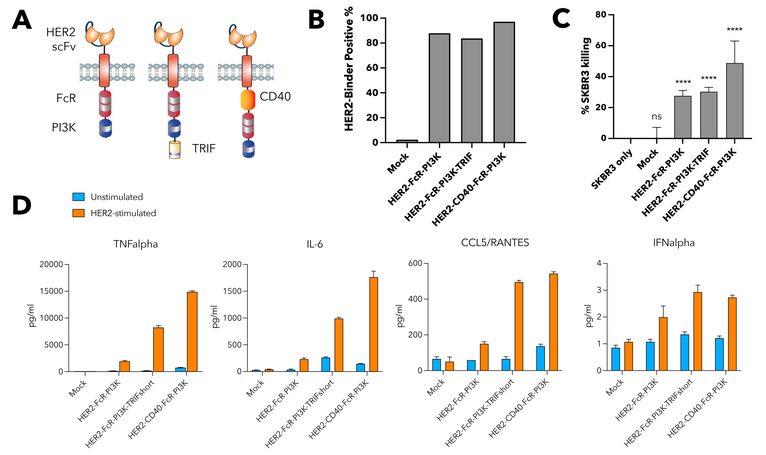


Figure 4: Primary monocytes armed with TRIF or CD40 based ATAK receptors kill tumor cells and produce pro-inflammatory cytokines and chemokines, *in vitro*. 2nd Gen ATAK receptors design (A) and expression in primary human monocytes using mRNA (B). Tumor killing activity of ATAK-monocytes after 3-day co-culture with HER2+ SKBR3 tumor cells (C). Antigen-specific proinflammatory cytokine expression in primary monocytes by TRIF and CD40 ATAK constructs.

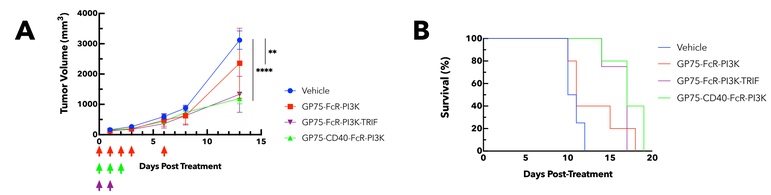


Figure 4: Primary monocytes armed with TRIF or CD40 based ATAK receptors showed strong anti-tumor response in B16 melanoma tumor model (A) Post-treatment melanoma tumor volume in mice treated with ATAK-monocytes. (B) Survival of tumor-bearing mice treated with FcR-PI3K ATAK receptor or ATAK receptors with TRIF/CD40 domains. Monocytes armed with 2nd Gen receptors showed increased potency, compared to monocytes armed with 1st Gen receptors.

Conclusions

- In a B16/F10 syngeneic model, mouse monocytes armed with TRIF or CD40 ATAK receptors exhibited strong anti-tumor efficacy, resulting in delayed tumor progression and significant prolonged survival
- 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.
- A clinical trial testing HER2 targeted 2nd Gen ATAK receptors is in development

References

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