ATYR2810 an anti-NRP2 monoclonal antibody targets Tumor Associated Macrophages

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Abstract

BACKGROUND: Neuropilin-2 (NRP2) is a single transmembrane pleiotropic receptor that utilizes co-receptors for signal transduction and is known to impact tumor progression and metastasis. Deletion of NRP2 in murine tumor-associated macrophages (TAMs) downregulated several immunosuppressive and tumor-promoting genes and upregulated immune-stimulatory genes in the myeloid compartment. However, little is known about the role of NRP2 in human TAMs. We previously reported significant expression of NRP2 on TAMs derived from triple negative breast cancer (TNBC), and demonstrated the ability of ATYR2810, a monoclonal anti-NRP2 antibody, to regulate epithelial-mesenchymal transition (EMT) genes, such as the transcription factor *ZEB1*, and to enhance chemotherapeutic efficacy for aggressive breast cancer. Knowing TAMs play an important role in EMT transition and therapy resistance of cancer, and also rely on *ZEB1* for their cancer promoting roles such as immune regulation, we sought to investigate the effects of ATYR2810 on TAMs.
METHODS: MDA-MB-231 TAMs were generated from monocytes in the presence or absence of ATYR2810. TAM phenotypes, gene expression and ability to secrete cytokines were assessed by flow cytometry, qRT-PCR and MSD respectively. TAM suppressive-ness was measured in co-culture experiments. T cell proliferation and activation markers were monitored by flow cytometry and cytokine production by MSD.
RESULTS: NRP2 is highly expressed on TAMs, which suppress T cell proliferation, activation and cytokine release. When differentiated in the presence of ATYR2810, a significant decrease in their suppressive capabilities against T cells was observed. Briefly, T cells were more proliferative, active and altered cytokine production when co-cultured with TAMs exposed to ATYR2810 compared to TAMs differentiated in its absence. Interestingly, we observed a significant decrease in *ZEB1* gene and protein expression in ATYR2810 treated TAMs compared to non-treated TAMs. ATYR2810 also decreased the suppressive ability of TAMs when present in co-culture experiments.
CONCLUSIONS: We show here for the first time that ATYR2810, known to bind NRP2 on tumor cells, can also bind and exert effects on human TAMs. Given the intricate relationship between TAMs and tumors, we believe that this novel finding provides additional insight into the mechanism of action of ATYR2810 as a potential immune regulator in cancer. We show for the first time that NRP2 has the ability to regulate *ZEB1* expression in TAMs; reducing their suppressive nature, pointing to a novel role of NRP2 in TAMs. These findings indicate ATYR2810's potential to be an effective anti-cancer agent, with one potential mechanism being through regulation of *ZEB1* in both TAMs and tumors.

Introduction

- NRP2 is a single transmembrane receptor, known to utilize VEGF receptors and plexins for signal transduction
- NRP2 has been shown to play a role in cell migration, antigen presentation, phagocytosis and cell-cell interaction within immune cells (Schellenburg et al, 2017)
- NRP2 has been described to play a role in the progression of tumors and their metastasis, but its contribution to this, and whether it shows immune regulation in the tumor microenvironment is still unknown (Caunt et al, 2008)
- NRP2 has been shown to be highly expressed on TNBC-derived TAMs (Tyler et al, 2021)
- *ZEB1* is an EMT marker, and has been shown to play a role in tumor progression via multiple mechanisms; ATYR2810 has been shown to down-regulate it in TNBC organoids (Xu et al, 2021)
- TAMs require *ZEB1* for their tumor-promoting and chemotherapy-resistance functions (Cortes et al. 2017)
- ATYR2810 is a monoclonal antibody developed by aTyr Pharma, that specifically binds and blocks the VEGF binding domain of NRP2

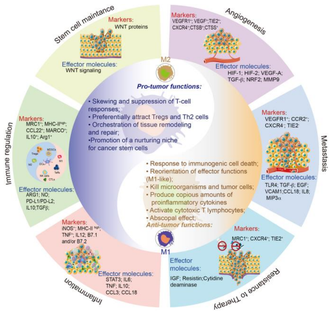


Fig 1. Schematic representation of TAM's roles in cancer.

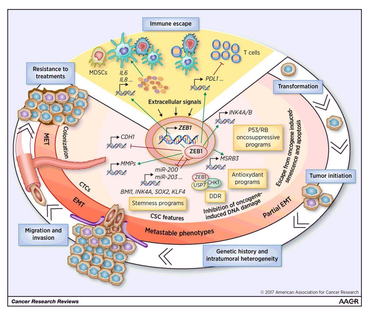


Fig 2. Schematic representation of ZEB1's roles in cancer.

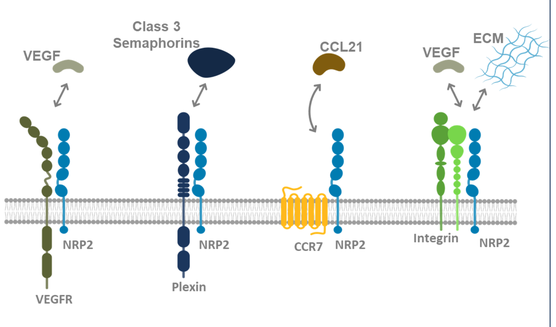


Fig 3. Schematic of NRP2 and its co-receptors and binding partners

Experimental Procedures

Fig 4. Generation of Primary Human MDA-MB-231 TAMs

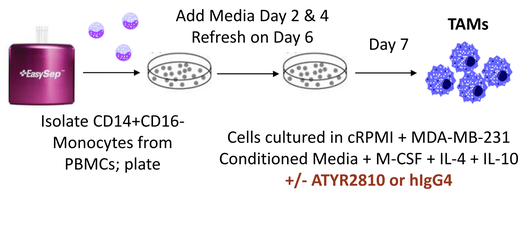
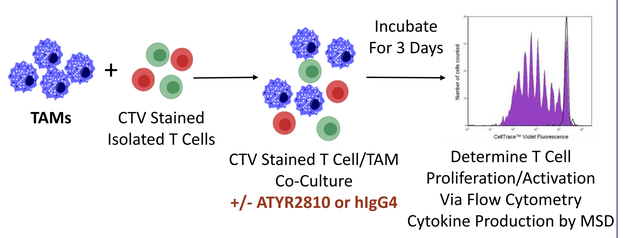


Fig 5. T Cell Suppression Assay



Figs 4-5. Schematic representation showing the experimental procedures to generate human TAMs from MDA-MB-231 conditioned media (Figure 4) and determine suppression of T cells by TAMs (Figure 5) . NRP2 levels, T cell proliferation and activation were measured using flow cytometry and analyzed using FlowJo. Cytokine levels were measured utilizing the Mesoscale Discovery UPLEX platform. Statistical analysis was performed using Prism.

Results

Fig 6. Immunophenotyping & NRP2 Expression on TAMs

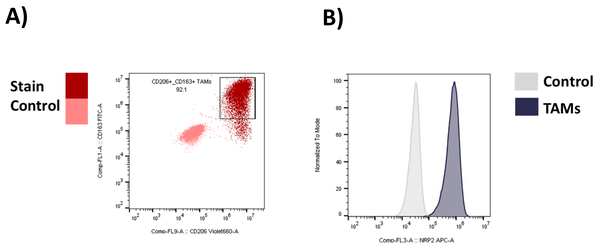


Fig 6. A) Dot plot representation of CD206+CD163+ TAMs generated as described in Fig. 4. **B)** Histogram representation of NRP2 expression detected on TAMs differentiated as described in Fig. 4

Fig 7. TAMs Ratio Dependently Suppress T Cells

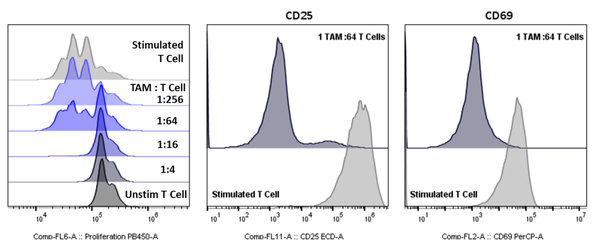
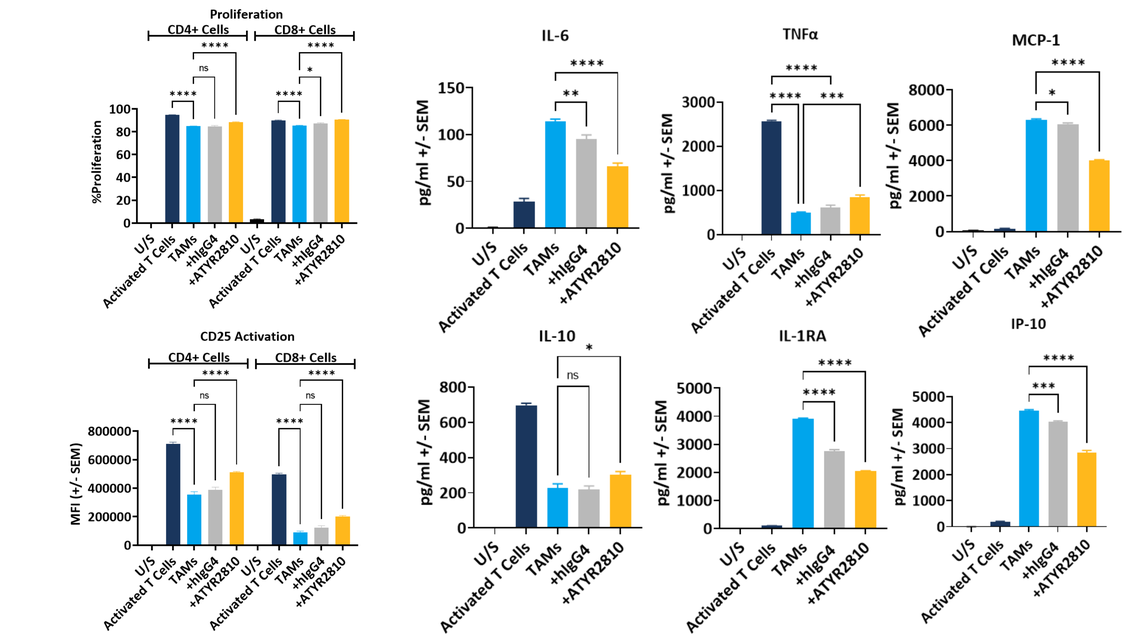


Fig 7. Histogram representation of TAM suppression of T cells shown by proliferation profiles, CD25 and CD69 expression.

Results

Fig 8. ATYR2810 Treated TAMs are Less Suppressive to T Cells

A) ATYR2810 Differentiated TAMs are less suppressive to T Cells



B) ATYR2810 treatment during T cell suppression assay reduces the supressiveness of TAMs

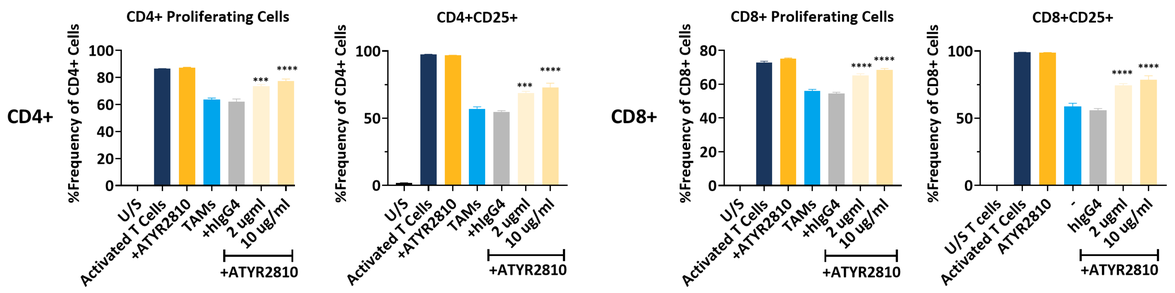
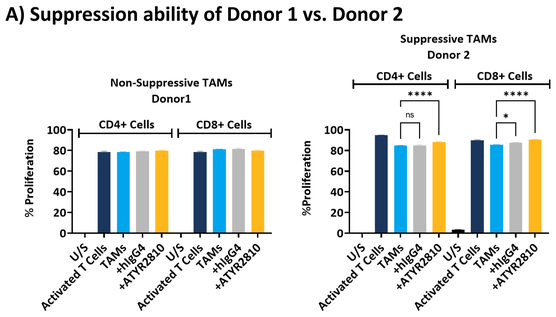


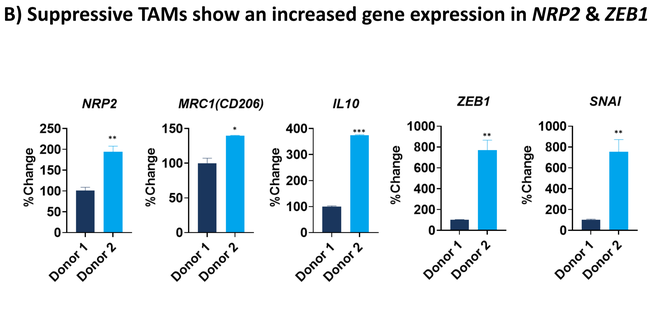
Fig 8. A) TAMs treated with 2810 during differentiation are less suppressive than untreated or hIgG4 treated TAMs, shown by an increase in % proliferating T cells, increased CD25 expression on T cells. Cytokine (IL-6, TNFα, IL10, IL1RA, IP-10) and Chemokine (MCP-1) are represented in graph format in the right panel. Graphs represent a single donor with significant effects seen in an n=3. **B)** ATYR2810 presence during co-culture of TAMs with T cells causes TAMs to be less suppressive of CD4+ T cells, shown by an increase in % proliferation of CD4+ T cells and % frequency of CD4/CD25 double positive T cells. Graphs represent a single donor with significant effects seen in an n=2.

Fig 9. ZEB1 Gene Expression Suppression as Potential Mechanism of Action

A) Suppression ability of Donor 1 vs. Donor 2



B) Suppressive TAMs show an increased gene expression in *NRP2* & *ZEB1*



C) ATYR2810 reduces the expression of *ZEB1*, *SNAI* & *SIRP1A*

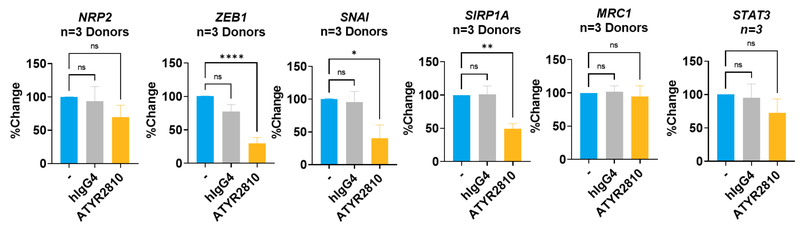


Fig 9. A) Graph representation of T cell proliferation ability of Donor 1 vs Donor 2. **B)** Gene expression profile Donor 1 vs Donor 2. Expression of *NRP2*, *MRC1*, *IL10*, *ZEB1*, *SNAI* are represented in bar graphs. **C)** Gene expression of *NRP2, ZEB1, SNAI, SIRP1A, MRC1 and STAT3* profile of TAMs generated from 3 separate donors in the presence or absence of either hIgG4 or ATYR2810.

Conclusions

- NRP2 has been shown to be highly expressed on CD163+CD206+ TAMs differentiated using MDA-MB-231 conditioned media and demonstrated to highly suppress T cell proliferation, activation and cytokine secretion
- TAMs differentiated in the presence of ATYR2810 are less suppressive to T cell proliferation, activation & cytokine production in comparison to TAMs differentiated in the absence of treatment or hIgG4 control
- ATYR2810 also altered the ability of TAMs to suppress T cell when treatment occurred during co-culture; ATYR2810 treatment of activated T cells in the absence of TAMs did not have an impact on proliferation or activation of T cells, indicating that ATYR2810 plays a direct role on TAMs
- Highly suppressive TAMs express high levels of *NRP2*, *ZEB1*, *IL10* and *SNAI* in comparison to TAMs that are not suppressive
- *ZEB1* and downstream gene *SNAI* were significantly reduced in ATYR2810 differentiated TAMs in comparison to NT or control TAMs, indicating that *ZEB1* might play an important role in regulating TAMs and their suppressive-ness
- Interestingly, we observed that ATYR2810 can also downregulate *SIRP1A*, but did not affect *STAT3, MRC1* or *NRP2* gene expression
- These findings indicate ATYR2810's potential to be an effective anti-cancer agent, with one potential mechanism being through regulation of *ZEB1* in both TAMs and tumors.

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