CD123+ plasmacytoid dendritic cells (pDCs) from systemic sclerosis patients are susceptible to the cytotoxic activity of tagraxofusp, a CD123-targeted therapy.

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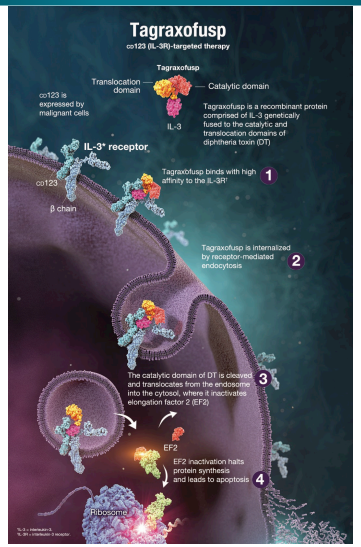
Background and Highlights

- Tagraxofusp was recently approved by the FDA, and is now commercially available in the U.S, for the treatment of patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN), a malignancy derived from the plasmacytoid dendritic cell (pDC) precursor.
- Tagraxofusp is a novel targeted therapy directed to the interleukin-3 receptor (CD123).
- Tagraxofusp is comprised of human IL-3 recombinantly fused to a truncated diphtheria toxin (DT) payload engineered such that IL-3 replaces the native DT receptor-binding domain. In this way, the IL-3 domain of tagraxofusp directs the cytotoxic DT payload to cells expressing CD123.
- Upon internalization, tagraxofusp irreversibly inhibits protein synthesis and induces apoptosis of the target cell.
- pDCs are immune cells that express CD123, secrete IFNα, and play a role in inflammation and disease pathogenesis observed in systemic sclerosis (SSc) and lupus patients[1,2,3].
- Therapeutic depletion of pDCs or attenuation of pDC function, may represent a novel approach to treating SSc patients.

Methods

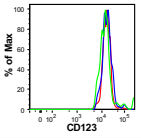
- Patients fulfilled the 2013 ACR/EULAR classification criteria for SSc[4]. peripheral blood mononuclear cells (PBMCs) from healthy volunteers (HV) and SSc patients were prepared using Ficoll-Paque density gradient from fresh blood.
- pDCs were isolated from PBMCs as previously described[5] and used to further enrich additional PBMCs.
- pDC-enriched PBMCs (3-6% pDCs) were cultured at $2x10^5$ cells per well in the presence or absence of CpG-274 (0.5 μM) to activate pDCs and then incubated with tagraxofusp (0.01-100 ng/ml, 0.17pM-1.7nM) at 37°C, 5% CO_2, and 95% humidity.
- After 24 h of culture, pDC survival was assessed by flow cytometry (CD14-, CD3- BDCA4+ CD123+), and supernatants were collected for cytokine quantification by a multiplexed Luminex assay.
- Changes in gene expression were measured by real-time quantitative PCR on 10ng cDNA, and calculated based on relative threshold cycle and expression of a ubiquitin housekeeping gene.

Tagraxofusp mechanism of action



Flow cytometry analysis of CD123 expression on pDCs of HVs and SSC patients

- CD123 expression on pDCs from healthy volunteers was compared to SSc patients by flow cytometry. MFI data shows a similar level of expression on samples tested.



Sample	CD123 MFI
HV	16501
SSc1	18852
SSc2	17886

Figure 1: Flow cytometry analysis of CD123 expression on pDCs from HV and SSc patients

Tagroxofusp is cytotoxic to pDCs from SSc patients

- $2x10^5$ pDC enriched PBMCs (3-6% pDCs) were cultured for 24hr with tagraxofusp (0.01-100 ng/ml, 0.17 pM-1.7 nM) in the presence or absence of CpG-274 (0.5 μM) for DC activation/maturation.
- pDC survival was assessed by flow cytometry (CD14-, CD3-BDCA4+ CD123+) and survival graphed against tagraxofusp concentration.
- Tagraxofusp was cytotoxic to pDCs from both HVs and SSc patients to a similar extent. ED_{50} of tagraxofusp in HVs and SSc patients was 4.3 and 3.2 ng/ml (74.4 and 55.4 pM) respectively.

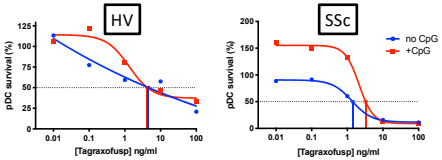


Figure 2: *Ex vivo* survival of pDCs from HVs and SSc patients treated with tagraxofusp. pDC enriched PBMCs were incubated with tagraxofusp for 24h and cell survival assessed by flow cytometry (n=5 HVs and n=5 SSc patients)

Tagraxofusp cytotoxicity is specific

- Following 24h culture with tagraxofusp, cell survival was assessed by flow cytometry on CD14+ monocytes, CD20+ B cells and CD4/8+ T cells.
- At high concentrations, tagraxofusp was cytotoxic to monocytes due to moderate expression of CD123 on monocytes[6].
- Tagraxofusp had no cytotoxic effect on B-cells or T-cells.

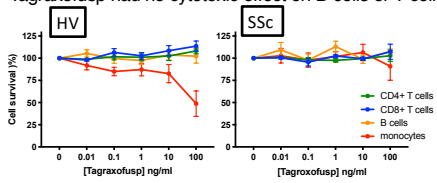


Figure 3: *Ex vivo* survival of monocytes, B cells and T cells from HVs and SSc patients treated with tagraxofusp. pDC enriched PBMCs were incubated with tagraxofusp for 24h and cell survival assessed by flow cytometry (n=5 HVs and n=5 SSc patients)

Tagraxofusp cytotoxicity results in a reduction of secreted IFNα and IFNα-induced gene expression

- The effect of tagraxofusp on CpG-induced cytokine secretion by PBMCs from SSc patients was assessed by screening cell culture supernatant by Luminex technology for IL-1β, IL-6, IL-8, IFNα, IFNγ IP-10, TNFα, MIP-1α/β, MCP1 and RANTES.
- A 68-fold reduction in CpG-induced IFNα protein secretion was observed in pDC-enriched patient PBMCs cultured with tagraxofusp; this was accompanied by a 3-fold reduction in the expression of the type 1 IFN-induced gene, GBP.

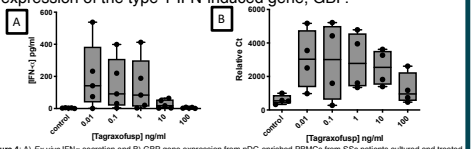


Figure 4: A) *Ex vivo* IFNα secretion and B) GBP gene expression from pDC-enriched PBMCs from SSc patients cultured and treated with tagraxofusp for 24h (n=5)

Conclusions and Next Steps

- CD123 expression on pDCs from HV and SSc patients is comparable.
- Tagraxofusp is cytotoxic against CD123+ pDCs from HVs and SSc patients.
- In SSc patients, pDC depletion by tagraxofusp was accompanied by a 68-fold reduction in CpG-induced IFNα protein secretion, and a 3-fold reduction in expression of the IFNα-induced gene, GBP.
- Tagraxofusp concentrations effective at eliminating inflammatory pDCs in this study were lower than peak plasma concentrations observed in BPDCN patients treated with the drug.
- These data present a potentially novel approach of targeting pDCs and inflammation in the treatment of SSc and warrant further investigation. A clinical trial is planned in autoimmune disease.

References

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