• 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 patients1,2,3.
• 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, 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 described4 and used to further enrich additional PBMCs.
• pDC-enriched PBMCs (3-6% pDCs) were cultured at 2x10⁵ 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, 0.17 μM-1.7 μM) at 37°C, 5% CO₂, and 95% humidity.
• After 24 h of culture, pDC survival was assessed by flow cytometry (CD14+, CD3- BDC-CD4+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.

Flow cytometry analysis of CD123 expression on pDCs of HVs and SSc patients
CDC132 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.

Tagraxofusp mechanism of action
Tagraxofusp is cytotoxic to pDCs from SSc patients
• 2x10⁵ pDC enriched PBMCs (3-6% pDCs) were cultured for 24h with tagraxofusp (0.01-100 ng/ml, 0.17 μM-1.7 μM) in the presence or absence of Cpg-274 (0.5 μM) for DC activation/maturation.
• pDC survival was assessed by flow cytometry (CD14+, CD3- BDC-CD4+CD123+), and survival graphed against tagraxofusp concentration.
• Tagraxofusp was cytotoxic to pDCs from both HVs and SSc patients to a similar extent. ED₅₀ of tagraxofusp in HVs and SSc patients was 4.3 and 3.2 ng/ml (74.4 and 55.4 μM) respectively.

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

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

CDC123 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.

Tagraxofusp cytotoxicity results in a reduction of secreted IFNα and IFNβ-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, IFNβ, IP-10, TNFα, 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.

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

CDC123 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.

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.
• Tagraxofusp had no cytotoxic effect on B-cells or T-cells.