Novel Interleukin-22 Surrogate Cytokine Agonists Improve Metabolic Syndrome

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ABSTRACT

Interleukin-22 (IL-22) is a pleiotropic cytokine that signals through the heterodimeric IL-22Rα:IL-10Rβ receptor complex. IL-22 exerts its activity predominantly on epithelial and epithelial-like cells (e.g. skin keratinocytes, gut/lung epithelium, hepatocytes), where it can promote mucosal barrier integrity, regulate tissue remodeling, and improve metabolic disorders[1]. However, IL-22-based therapeutics may be susceptible to silencing from IL-22 binding protein (IL-22BP) – an endogenous soluble antagonist that can inhibit the protective activity of IL-22 in disease. In addition, WT IL-22 induces proinflammatory cytokines and dose limiting skin inflammation in patients[2].

Here we report on novel IL-22 surrogate cytokine agonists (IL-22 SCAs). These IL-22 SCAs elicited receptor activation and avoided binding to IL-22BP. Selected IL-22 SCAs demonstrate efficacy by promoting improved weight and metabolic outcomes in mice and in protecting against colitis (data not shown).

A panel of 160 murine IL-22 SCAs were screened for IL-22R-dependent STAT3 phosphorylation activity and evaluated for binding to IL-22BP. Selected half-life extended, mouse-specific SCAs were tested for efficacy in a diet-induced obesity (DIO) model and in a DSS-colitis model. Effects on histopathology, metabolic hormones, proinflammatory cytokines/chemokines, glucose, insulin, liver triglycerides, and liver enzymes were monitored.

Murine IL-22 SCAs showed a range of pSTAT3 activity and lacked measurable binding to IL-22BP. Half-life extended IL-22 SCAs had a high and durable exposure in DIO mice, leading to robust weight reduction and hypophagia, associated with increased expression of satiety hormones and improvement of hyperglycemia and hyperinsulinemia. Additionally, IL-22 SCA treatment showed protection against DSS-induced colonic inflammation. Importantly, while IL-22 WT induced proinflammatory cytokines systemically, the IL-22 SCAs avoided this induction.

IL-22 SCAs improved metabolic syndrome in DIO mice with a sustained reduction of body weight, protected against DSS colitis (data not shown), and avoided inducing signs of systemic inflammation associated with IL-22 WT treatment.

IL-22 WT induces pSTAT3 & improves metabolic syndrome, but causes skin inflammation

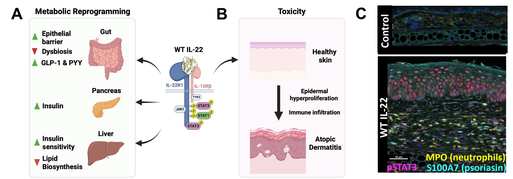

Figure 1: WT IL-22 is a critical regulator of epithelial cell homeostasis via STAT3 signaling Schema of the effects of IL22-WT on **A**: gut, pancreas, liver and **B**: keratinocytes. **C**: Expression of inflammatory markers in the skin of obese mice chronically treated with mIL-22 WT (bottom). **D**: Relative expression of IL-22Rα & IL-10Rβ mRNA in mouse duodenum, colon, or skin cells.

Surrogate Cytokine Agonist: a modular VHH-based platform for activating cytokine receptor signaling

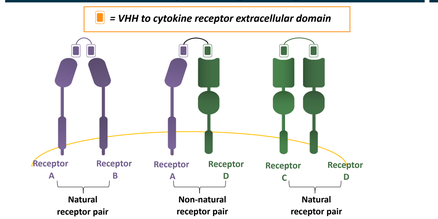

Figure 2: Surrogate Cytokine Agonist (SCA)
- Surrogate Cytokine Agonists (SCAs) represent a novel cytokine engineering approach using synthetic ligands (linked VHH) *in lieu* of modified cytokines
- VHHs are single domain antibodies isolated from camelids
- Non-natural pairing can access unique biology or target specific cell subtypes
- Intracellular signaling can be tuned by altering proximity or geometry of the dimerized cytokine receptors
- Potential for an almost unlimited array of biased signaling possibilities

IL-22 SCAs avoid IL-22BP binding, have high durability and a range of functional phenotypes

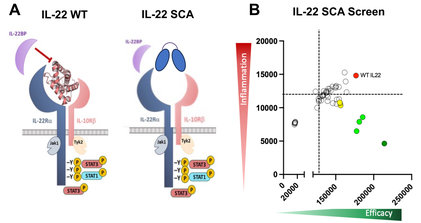

Figure 3: IL-22 SCAs evade IL-22 BP binding and have a range of functional phenotypes. Intrinsically, skin cells express less IL-22Rα/IL10Rβ
A: Binding of WT IL-22 vs IL-22 SCA to receptors. **B:** Screen of IL-22 SCA panel (dotted lines represent untreated).

Mouse IL-22 SCAs are active on mouse colon epithelial cells, not on keratinocytes

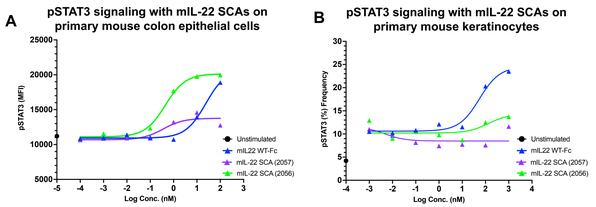

Figure 4: pSTAT3 induction on GI epithelial cells by mouse IL-22 SCAs and have reduced pSTAT3 activity in primary mouse keratinocytes A: pSTAT3 induction in primary mouse colon epithelial cells after 20 mins stimulation with mIL-22 SCAs *in vitro*; **B:** pSTAT3 induction in primary mouse keratinocytes after 20 mins stimulation mIL-22 SCAs *in vitro*.

mIL-22 SCAs significantly limited induction of skin inflammation, hyperplasia, and neutrophil infiltration

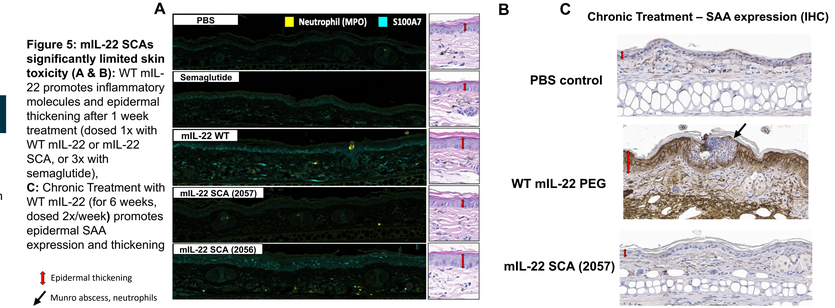

Figure 5: mIL-22 SCAs significantly limited skin toxicity (A & B): WT mIL-22 promotes inflammatory molecules and epidermal thickening after 1 week treatment (dosed 1x with WT mIL-22 or mIL-22 SCA, or 3x with semaglutide), **C:** Chronic Treatment with WT mIL-22 (for 6 weeks, dosed 2x/week) promotes epidermal SAA expression and thickening

mIL-22 SCAs induce durable body weight reduction in DIO mice

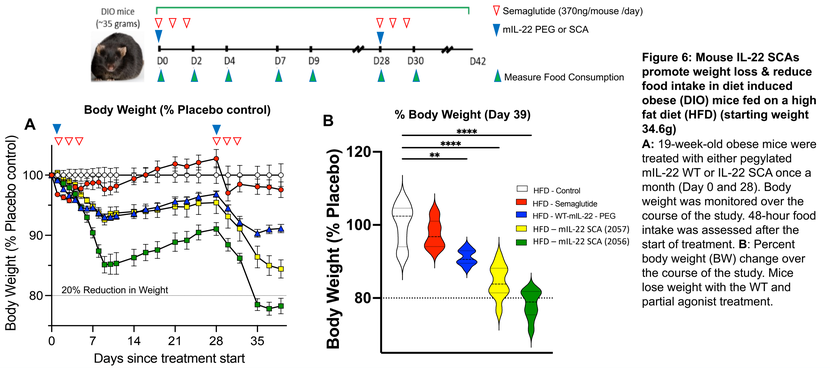

Figure 6: Mouse IL-22 SCAs promote weight loss & reduce food intake in diet induced obese (DIO) mice fed on a high fat diet (HFD) (starting weight 34.6g)
A: 19-week-old obese mice were treated with either pegylated mIL-22 WT or IL-22 SCA once a month (Day 0 and 28). Body weight was monitored over the course of the study. 48-hour food intake was assessed after the start of treatment. **B**: Percent body weight (BW) change over the course of the study. Mice lose weight with the WT and partial agonist treatment.

IL-22 SCAs combine with semaglutide to enhance fat reduction

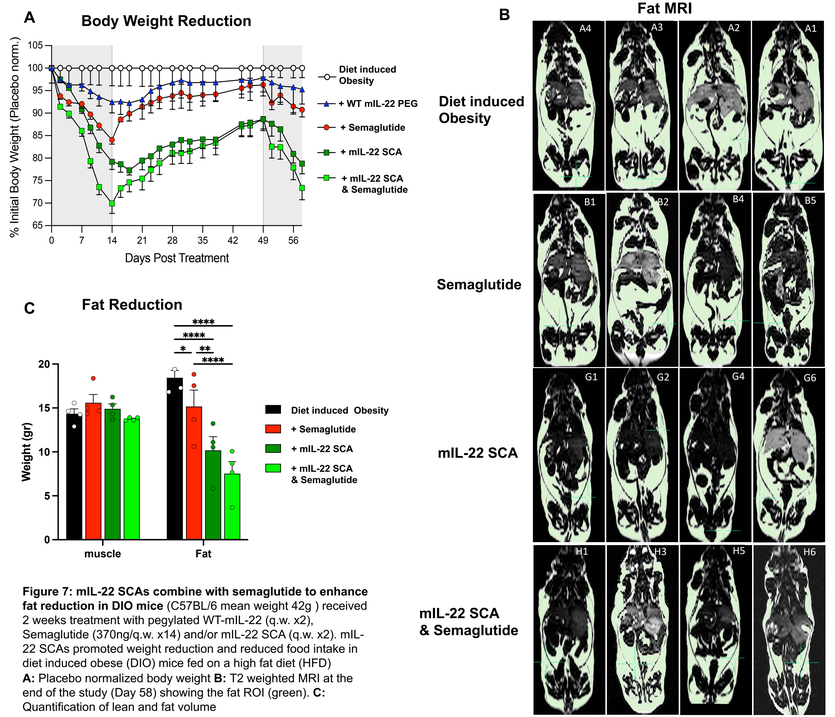

Figure 7: mIL-22 SCAs combine with semaglutide to enhance fat reduction in DIO mice (C57BL/6 mean weight 42g) received 2 weeks treatment with pegylated WT-mIL-22 (q.w. x2), Semaglutide (370ng/q.w. x14) and/or mIL-22 SCA (q.w. x2). mIL-22 SCAs promoted weight reduction and reduced food intake in diet induced obese (DIO) mice fed on a high fat diet (HFD)
A: Placebo normalized body weight **B:** T2 weighted MRI at the end of the study (Day 58) showing the fat ROI (green). **C:** Quantification of lean and fat volume

Summary

IL-22 surrogate cytokine agonists induce weight loss and metabolic reprogramming without inducing skin toxicity

1. **IL-22 is a unique cytokine that predominantly acts on epithelial cells and has been shown to improve metabolic disorders. However, because of its pleiotropy, WT IL-22 also causes dermatological toxicity.**
2. **mIL-22 SCAs are mIL-22Rα and mIL-10Rβ binders that are generated to decouple improvement in metabolic disorders from dermatological toxicity.**
3. **mIL-22 SCA treatment of DIO mice reduces body weight, associated with increase in satiety hormones (data not shown) while avoiding skin toxicity (hyperplasia, and inflammatory activity) associated with IL-22 WT treatment.**
4. **mIL-22 SCAs combine with semaglutide to reduce weight.**
5. **mIL-22 SCAs are active on human colon epithelial cells and show reduced activity on human keratinocytes.**

1.) Wang X, *et al*. Interleukin-22 alleviates metabolic disorders and restores mucosal immunity in diabetes. Nature. 2014; 514(7521):237-41
2.) Saxton RA, *et al*. The tissue protective functions of interleukin-22 can be decoupled from pro-inflammatory actions through structure-based design. Immunity. 2021; 54(4): 660-672