Preclinical Investigations of IMU-838, an Orally Available Small Molecule Inhibitor of Dihydroorotate Dehydrogenase for the Treatment of Inflammatory Bowel Disease

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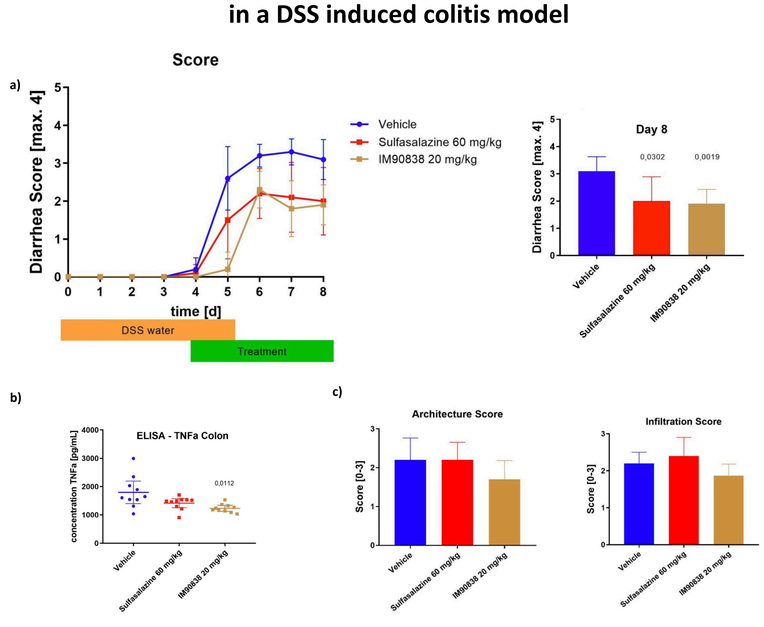
Background

In addition to the currently available treatment options for inflammatory bowel disease, there is a need for safe and easy to use oral drugs. IMU-838 is an orally available, small molecule Dihydroorotate Dehydrogenase (DHODH) inhibitor which demonstrated good tolerability in more than 500 individuals treated so far. The inhibition of DHODH by small molecules provides a natural selectivity towards hyperactivated immune cells, without providing a general immunosuppressive action Klotz et al., *Sci. Transl.Med.*11, 2019. Currently, three phase 2 studies are ongoing with IMU-838 in multiple sclerosis, primary sclerosing cholangitis and UC (ulcerative colitis). In the UC phase 2b study, IMU-838 is administered using 10, 30, 45 mg of daily dosing. An interim dosing analysis demonstrated that all three doses are well tolerated and that the lowest dose of 10 mg is likely not to be ineffective.

This poster provides data with regard to low dose activity in an DSS induced colitis model, species selectivity towards the target DHODH, impact on metabolically highly activated lymphocytes and induction of treatment relevant regulatory macrophages in combination with anti-TNF-α treatment.

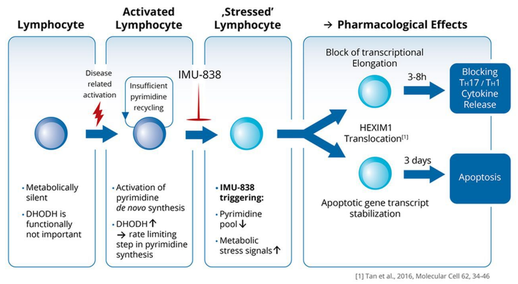
Low dose of IMU-838 demonstrates therapeutic activity in a DSS induced colitis model



Method: C57BL/6 mice were challenged with 2.8% DSS from day 0 to day 5 and were treated with vehicle, 60mg/kg Sulfasalazine or 20mg/kg IMU-838 by gavage from day 4 to day 8 (n=10 per group). Read-out: Daily diarrhea score. At day 8, mice were sacrificed and colonic TNF-α levels were assessed by ELISA and a tissue architecture score and immune cell infiltration score were determined by histology.

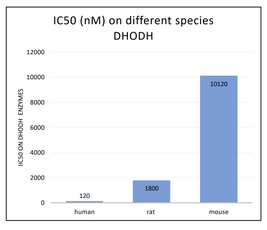
Result: a) Low dose (20mg/kg) of IMU-838 improved the symptoms of colitis as depicted by the diarrhea score. b) IMU-838 reduced expression of pro-inflammatory TNF-α in the colon. c) Additionally, IMU-838 slightly improved the histological architecture of the gut wall and reduced immune cell infiltration. In previous experiments, much higher doses of IMU-838 were used (50-200mg/kg) to investigate activity. Median plasma concentration of IMU-838 free acid equivalent at 3h after last treatment was 310ng/ml. (Conc. at T_{max} (1h) not determined due to experimental set-up)

IMU-838 induces metabolic stress in highly activated immune cells



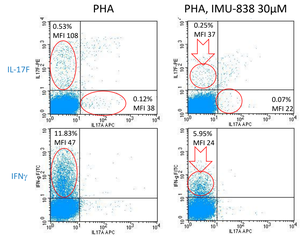
Model: Targeting DHODH with IMU-838 provides a natural selectivity towards metabolically highly activated lymphocytes, like hyperactivated T cells in a chronic inflammatory setting. DHODH inhibition leads to a metabolic stress signal, mediating an immediate repression of pro-inflammatory cytokines like IL-17 and IFN-γ, as well as inducing T cell apoptosis when metabolic stress signal holds on.

IMU-838 is most potent on human DHODH and targets preferentially high producing IL-17 and IFN-γ cells



Method: Human, rat and mouse DHODH enzymes were overexpressed in BL2-RiL E.coli and purified. IMU-838 free acid equivalent was tested for its inhibitory capacity *in vitro*. Read out: DHO oxidation, electron transfer to Dichlorindophenol (DCIP), absorption at 600 nm.

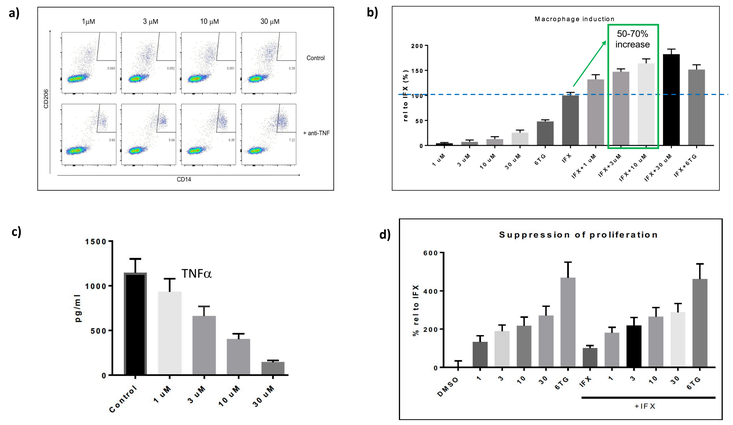
Result: IMU-838 shows a strong species selectivity. IMU-838 free acid equivalent is most potent on human DHODH with an IC50 of 120nM, followed by the rat DHODH with an IC50 of 1800nM, whereas it is ~84 times less effective on murine DHODH.



Method: Human PBMCs were stimulated *ex vivo* with 2µg/ml PHA for 20h and treated with 30µM of IMU-838 free acid equivalent. Cells were stained intracellularly for IL-17A, IL-17F and IFN-γ and analyzed by FACS.

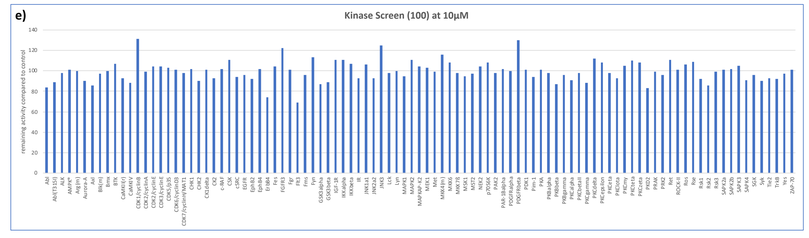
Result: IMU-838 mainly reduces the amount of high producing IL-17A, IL-17F and IFN-γ cells, whereas the lower producing cells are still present. Results shown are representative of n=3 independent experiments. Assay duration of 48h leads to IC50 values for IL-17 and IFN-γ of 3-5µM (ELISA, data not shown).

IMU-838 and infliximab synergistically induce regulatory macrophages and IMU-838 suppresses T cell proliferation without targeting kinases



Method: In a mixed lymphocyte reaction assay (MLR), PBMCs of two donors were incubated for 48h and subsequently treated with IMU-838 or/and infliximab (IFX) for 4 days (n= 3 independent experiments, each measured five-fold). Regulatory macrophages ($CD14^+CD206^+$) were determined by FACS analysis. T cell proliferation was determined by 3H Thymidine incorporation. Secreted TNF-α was determined by Cytometric bead analysis.

Result: a) IMU-838 induces small numbers of CD14+CD206+ macrophages compared to IFX, but largely increases the induction of regulatory macrophages when in the presence of IFX (b). c) TNF-α secretion and d) T cell proliferation are strongly suppressed by IMU-838 treatment.



Method: Kinase screen was performed with 100 human kinases (exceptions marked – murine (m), rat (r)) at 10µM concentration of IMU-838 free acid equivalent. ATP conc. was 100µM.

Result: Inhibition of kinases is depicted as % remaining activity compared to negative control. IMU-838 free acid equivalent does not hit any kinases with more than 30% inhibition at physiological relevant concentrations (10µM).

Summary & Conclusion

IMU-838 has demonstrated activity at doses lower than previous used in a preclinical *in vivo* model of DSS induced colitis. This activity is likely to be mediated by a direct impact on metabolically hyperactivated T lymphocytes in the gut, by reducing IL-17 and IFN-γ high producing cells, by the reduction of TNF-α, as well as the inhibition of T cell proliferation and induction of T cell apoptosis (data not shown). IMU-838 seems to lack off-target effects on the large range of kinases investigated in this study. This would hypothesize a safety profile potentially lacking any adverse effects known to be associated by effects on kinases, such as cell growth inhibition in relation to EFGR inhibitory activity Mattar et al., FEBS Lett. 1993. In a preclinical combination study with anti-TNF treatment, IMU-838 acted synergistically on the induction of disease modifying regulatory macrophages. IMU-838 is currently tested in a phase 2b trial in UC patients in monotherapy. In addition to this preclinical data support a potential combination trial with anti-TNF treatments. The hypothesis for this is that a combined treatment of UC patients with IMU-838 and e.g. infliximab could potentially reduce the percentage of anti-TNF-α non-responders or re-activate responses towards anti-TNF-α treatments by inducing regulatory macrophages.