Abstract citation ID: izac247.0146

SUBCUTANEOUS INFLIXIMAB IN REFRACTORY CROHN'S DISEASE PATIENTS AFTER THE MULTIPLE TREATMENT FAILURES - SHORT TREATMENT OUTCOMES

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BACKGROUND: Subcutaneous administration of infliximab shows an increased benefit in terms of pharmacokinetics and pharmacodynamics effects compared to the intravenous infliximab.

The practical question arises whether routine administration in refractory CD patients who have failed prior biological therapy will provide "biobetter" clinical efficacy.

AIMS: We propose a single center study designed to evaluate SC-IFX treatment outcomes in patients with active CD after the previous failures of other biologicals (TMAbs). Such a refractory CD cohort was chosen because there is currently insufficient appropriate medication therapy available for these patients and the identification of parameters predicting response to SC-IFX treatment could be of great benefit to some of them.

PATIENTS & METHODS: A total of 32 CD patients were included, with the median age of 34.5 years and median CD duration of 11 years; >90% of them with stricturing and/or penetrating disease behavior, >80% with ileocolic involvement, and about 40% with the active perianal disease. Eighteen of 32 patients (56%) have failed on ≥3 previous TMAbs, the rest on 2 previous TMAbs. Twenty (62.5%) of n = 32 have had IV-IFX in past; in this sub-cohort, 85% of patients have positive anti-IFX antibodies (ATI). In ATI-positive sub-cohort, induction of SC-IFX was realized with four 120 mg SC doses weekly. Two induction IV infusions of 5 mg/kg IFX were administered in 2-week intervals in ATI-negative sub-cohort. Maintenance treatment in whole cohort consisted of 120 mg SC IFX bi-weekly.

RESULTS: Of the 32 patients, 7 individuals have failed the treatment during the induction phase: one because of heart disease progression, and 6 patients due to delayed hypersensitivity reactions with systemic manifestation. In 25/32 patients who continued their treatment up to W14, the initial median Harvey-Bradshaw index (HBI) of 6 (min 1, max 28) did not decrease significantly within the 14 weeks of treatment (p=0.067). However, both the serum CRP level (p=0.039) and fecal calprotectin level (p=0.027) have shown significant decrease. Median trough IFX levels have increased from 6 µg/mL at W2 to 10 µg/mL at W14 (p<0.001), and median ATI levels dropped from median of 38 ng/mL to 5 ng/mL (p= 0.0004). None of the patients with initial zero ATI levels have shown new ATI formation. Moreover, in 10 of 17 patients (59%) with the initial ATI positivity, ATI serum levels have decreased significantly even to the cut-off levels. The only risk factor for SC-IFX discontinuation before W14 was a high serum levels of ATIs (p < 0.001).

CONCLUSIONS: Our short-term experience with SC-IFX shows the treatment efficacy and tolerability in complicated CD patients after multiple TMAbs failures.The research gap in information about biobetters in patients with IBD must be filled and predictive factors for the response to SC-IFX must be found.

Translational Application of Intestinal Stem Cells and Organoid Models

Abstract citation ID: izac247.0147

EICOSATETRAYNOIC ACID REGULATES PRO-FIBROTIC PATHWAYS IN AN INDUCED PLURIPOTENT STEM CELL DERIVED MACROPHAGE:HUMAN INTESTINAL ORGANOID MODEL OF ILEAL CROHN'S DISEASE

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INTRODUCTION: Biologics targeting TNF are the mainstay of therapy for children with Crohn's Disease (CD). However, a subset of patients do not respond, progressing to intestinal fibrosis requiring surgical resection. Prior studies have defined an ileal gene expression module linked to future strictures, and identified small molecules which may reverse this gene signature and suppress fibroblast activation. In the current study we developed a pre-clinical model system and tested a lead candidate, eicosatetraynoic acid (ETYA), a Peroxisome Proliferator-Activated Receptor (PPAR) agonist and arachidonic acid metabolism inhibitor.

METHODS: Peripheral blood samples were collected from pediatric CD patients and induced pluripotent stem cell (iPSC) lines were generated. iPSC were differentiated into human intestinal organoids (HIOs) and macrophage-like cells. Macrophage:HIO co-cultures were exposed to lipopolysaccharide (LPS) with and without eicosatetraynoic acid (ETYA) pre-treatment. Flow cytometry and cytospin characterized macrophage activation markers and morphology. Co-culture populations were harvested, and RNA and conditioned media were isolated for downstream analysis. TaqMan Low Density Array, Luminex multiplex assay, immunohistologic staining, and sirius red polarized light microscopy were performed to quantify measures of inflammation and fibrosis, and to test whether introduction of ETYA abated any of these inflammatory or fibrotic changes.

RESULTS: iPSC-derived macrophages exhibited morphology similar to primary macrophages, and expressed inflammatory macrophage cell surface markers including CD68 (Fig. 1A). LPS-stimulated iPSC-derived macrophages expressed a global pattern of gene expression by RNA sequencing enriched in CD ileal inflammatory macrophages (ToppCell Atlas, p=4.397E-117), and produced cytokines and chemokines implicated in refractory disease (Fig. 1B). Co-culture of LPS-primed macrophages with HIO led to up-regulation of the fibroblast activation genes ACTA2 and COL1A1 (Fig. 2). Under these conditions, HIO collagen content measured by sirius red staining and polarized light microscopy was increased (Fig. 2). ETYA pre-treatment prevented these pro-fibrotic effects of LPS-primed macrophages upon HIO gene expression and collagen content. However, LPS induction of macrophage IL1B, TNF, and OSM production was not suppressed by ETYA, suggesting an alternative mechanism of action upon HIO fibroblast activation and collagen content in the co-culture system.

CONCLUSIONS: ETYA inhibits effects of LPS-primed macrophages upon HIO pro-fibrotic gene expression and collagen production. This was not associated with an effect of ETYA upon macrophage inflammatory cytokine production. Future studies will test alternate pathways including PPAR activation and arachidonic acid metabolism which may mediate this response.

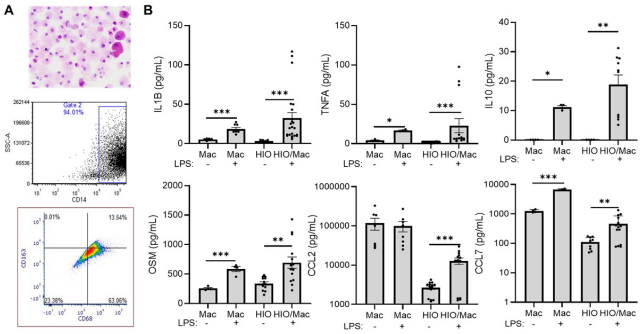

Figure 1. **iPSC-derived macrophages exhibit a pro-inflammatory pattern of cytokine and chemokine production implicated in anti-TNF non-response.** A) Induced pluripotent stem cells (iPSC) were differentiated into macrophage-like cells over 25 days. Cell morphology was assessed using cytospin, and CD14 expression, and CD68 and CD163 expression on CD14+ cells, was determined using flow cytometry. Representative images of scatter plots are shown, n=14. B) iPSC-derived macrophages (Mac) or human intestinal organoids (HIO) were assayed under basal conditions, or following macrophage:HIO (HIO/Mac) co-culture ± LPS, 100 ng/mL for 72 hours. Cytokine and chemokine secretion was measured using a luminex assay. Data are shown as the mean (SEM), n=4-14 per group, *p<0.001.

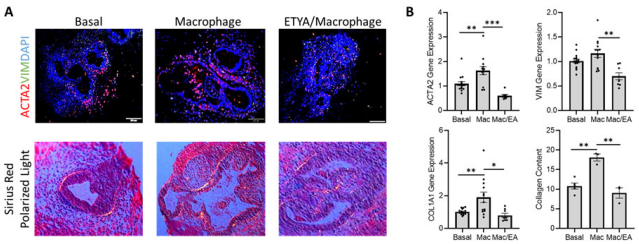

Figure 2. **Eicosatetraynoic acid (ETYA) prevents up-regulation of HIO ECM gene expression and collagen content by LPS primed macrophages in co-culture.** Human intestinal organoids (HIOs) were studied under basal conditions, with LPS primed macrophage (Macrophage/Mac) 72 hour co-culture, or following ETYA exposure, 50 mM for 14 days, preceding LPS primed macrophage 72 hour co-culture (ETYA/Macrophage & Mac/EA). A) Representative images are shown for alpha smooth muscle actin (ACTA), vimentin (VIM), and DAPI staining, and for Sirius red staining with polarized light microscopy (arrow). B) Human intestinal organoid ACTA2, VIM, and collagen (COL1A1) gene expression were determined by PCR. Collagen protein was detected in HIO using Sirius red staining and polarized light microscopy (arrows). Organoid collagen content was quantified using ImageJ. Data are shown as the mean (SEM), n=9-16 per group for PCR and collagen content data,*p<0.001.