Evaluation of IL23R as a target for CAR-Tregs at the site of inflammation in subjects with Crohn's Disease

Poster P487

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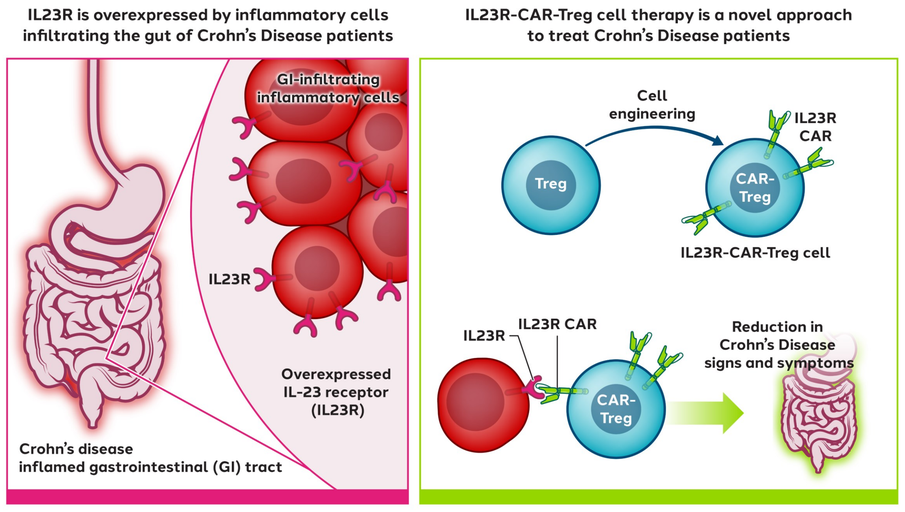
Introduction

Crohn's disease (CD) is a chronic inflammatory bowel disease characterized by uncontrolled immune responses in the gastrointestinal tract. By targeting a disease-related protein, regulatory T cells (Tregs) engineered with a chimeric antigen receptor (CAR-Tregs) represent a potential therapeutic alternative for treating CD. The role of interleukin 23 (IL-23) and its receptor IL23R in the pathogenesis of CD is well documented (Duerr et al., 2006, Neurath et al., 2019). We therefore developed a CAR-Treg targeting IL23R.

- Our IL23R-CAR-Tregs have very low tonic signaling and a desirable signal-to-noise ratio
- Moreover, IL23R-CAR-Tregs exhibited IL23R-dependent suppressive activity *in vitro* and in mice with dextran sodium sulfate (DSS)-induced colitis



Here, we show that:

- Immunohistochemical analysis of intestinal biopsies from CD subjects showed that mucosal lamina propria cells express IL23R
- Using biopsy-derived intestinal cells from CD patients, we observed IL23R-specific CAR-Treg activation towards colonic cells mainly from severe CD patients, also from mild-moderate CD patients to a lesser extent
- Finally, we performed molecular profiling of CD patients to support clinical development

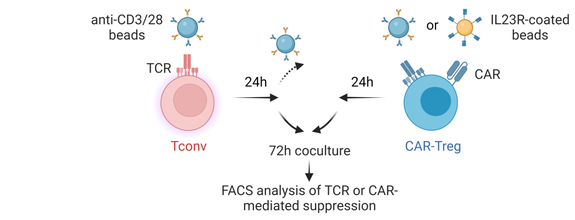
Overall, we validated IL23R as a target and its specificity to induce IL23R-CAR-Treg activation at the site of inflammation in CD patients, which warrants further investigation in clinical trials.

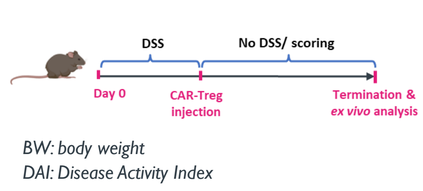
Results

1 IL23R-CAR-Tregs exhibit IL23R-dependent suppressive activity *in vitro* and in mice with colitis

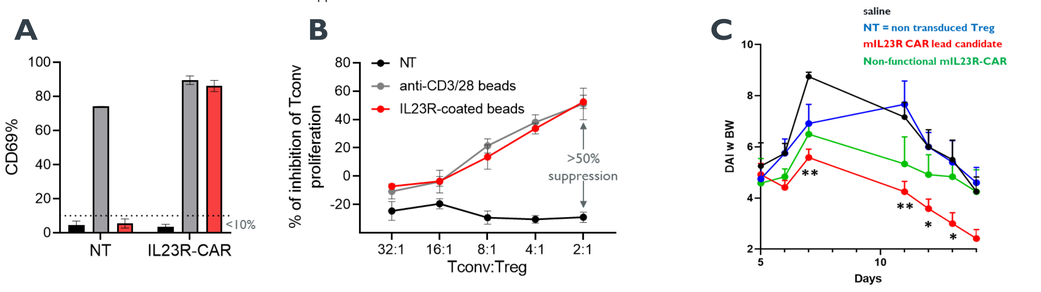
IL23R-CAR-Tregs exhibited **(A)** very low tonic signalling, a desirable signal-to-noise ratio, and **(B, C)** IL23R-dependent suppressive activity *in vitro* and in mice with DSS-induced colitis.

Previous poster available at https://vu.fr/gJoOp





BW: body weight
DAI: Disease Activity Index



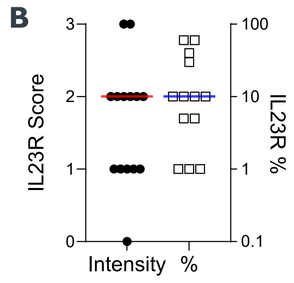
2 Histology analysis validated the target IL23R expression in intestinal biopsies from CD patients

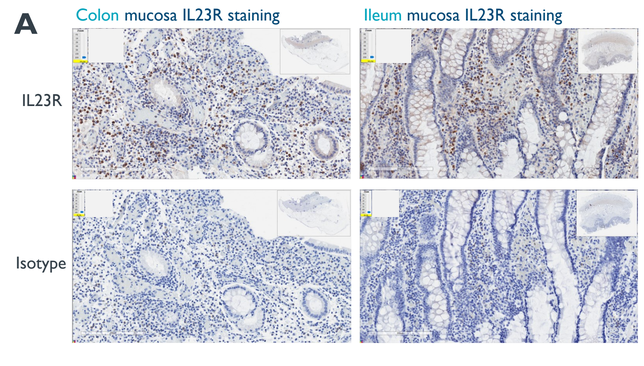
IL23R staining of the colon and ileum mucosa displayed active CD features:

(A) IL23R staining of representative colon or ileum sections using commercial antibody

(B) Pool of 15 samples from 10 moderate-severe CD subjects

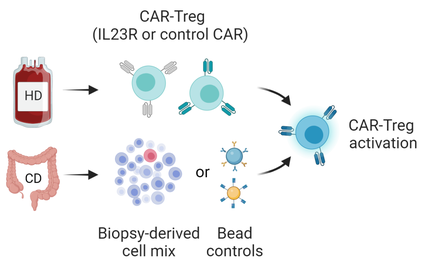
The expression of IL23R was also verified using IL23R-CAR binding domain, please refer to poster **P503** for details.



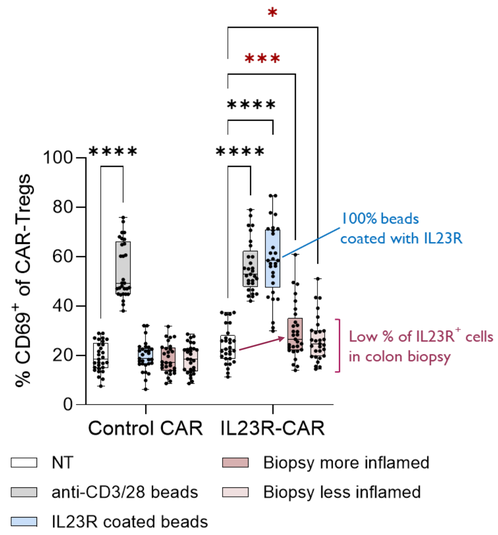


3 IL23R CAR-Tregs respond to intestinal cells from CD patients in an IL23R-specific manner

Tregs isolated from healthy donors (HD) were transduced with IL23R or control CAR constructs and cultured for 10-11 days. Freshly isolated colonic cells from CD patients were used as the source of antigen-expressing cells for Treg activation assay.

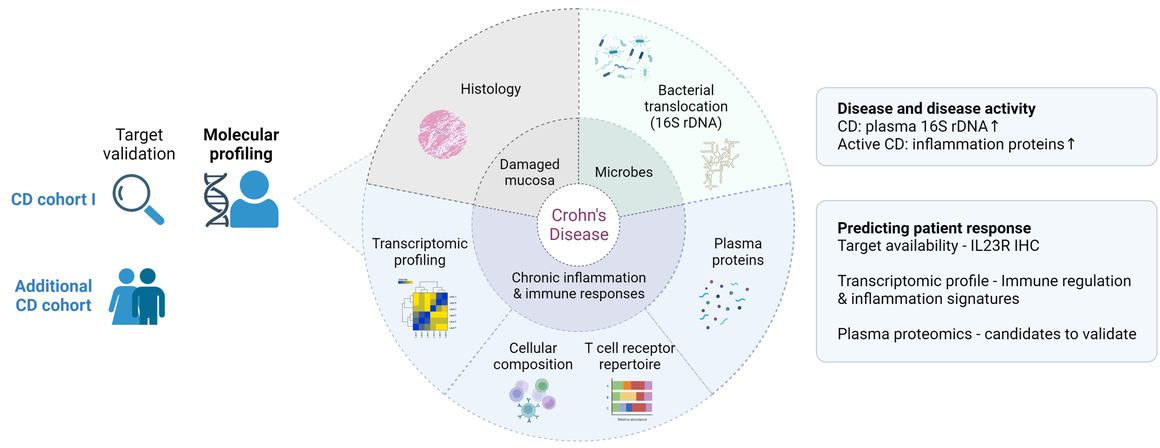


Activation of IL23R CAR-Tregs after overnight coculture with colon biopsy cell mixture from severe CD patients. Anti-CD3/CD28 beads and IL23R coated beads were used as positive controls, culture media alone (NT) as negative control. Each dot represents a Treg donor. Similar results were observed with GARP (data not shown).



4 Certain molecular profiles are associated with IL23R-CAR-Treg activity

Overview: molecular profiling of CD patients from cohort I



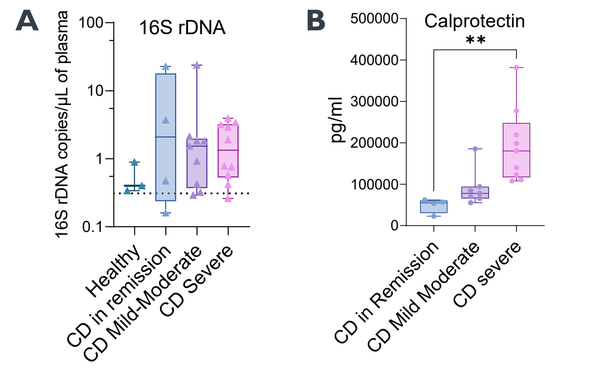
(A) An increase of plasma 16S rDNA was observed in CD patients, regardless of disease stage.

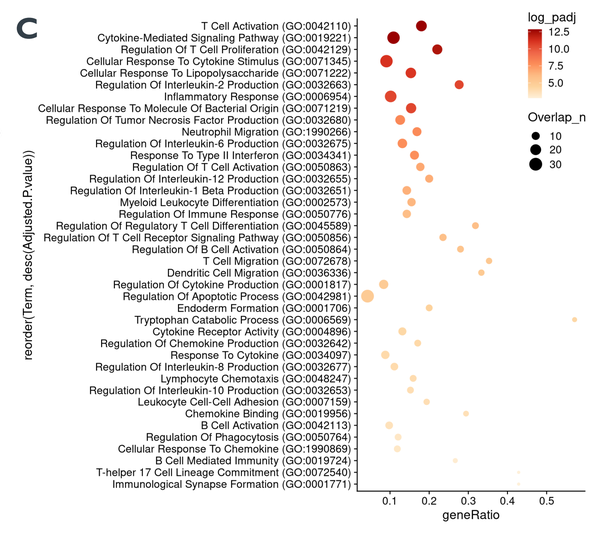
(B) Calprotectin concentration increased in active CD patients.

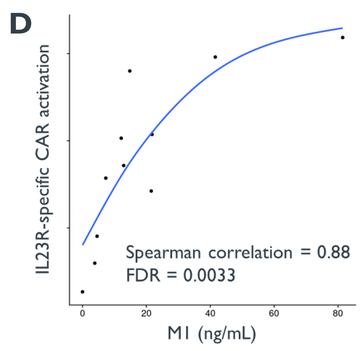
Transcriptomic profiles and plasma proteins positively correlated with patient biopsy-induced IL23R-specific CAR activation.

(C) Enrichment analysis of positively correlated genes is performed using EnrichR. Top 40 ranked Gene Ontology (GO) terms according to adjusted p-value are displayed here. Log_padj, log 10 of the adjusted p value. Overlap_n, number of enriched genes in a GO term. Gene ratio, percentage of enriched genes in the given GO term

(D) Correlation analysis between plasma M1 concentration and patient biopsy-induced IL23R-specific CAR activation. Piecewise cubic spline (solid blue line) is used to approximate the relationship between the two variables.



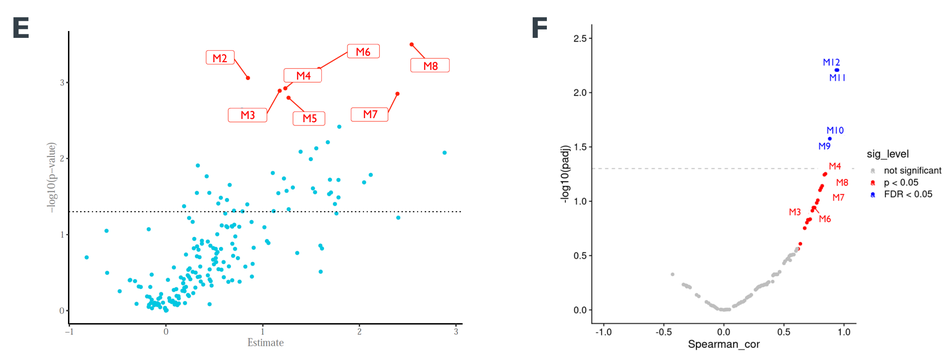




Plasma protein screening using Olink Proximity Extension Assay (PEA) technology

(E) Volcano plot of the Group strong vs Group weak/no IL23R-specific CAR activation. The dotted line represents the uncorrected significance threshold of 0.05. The x-axis depicts the Normalized protein expressions (NPX) difference between the groups for each protein measured.

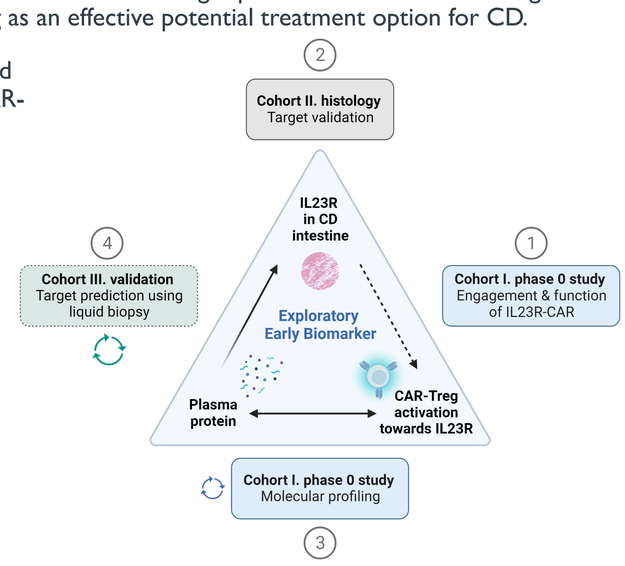
(F) Correlation analysis between Olink NPX and patient biopsy-induced IL23R-specific CAR activation. The dotted line represents the FDR threshold of 0.05



Conclusions and perspectives

Using primary cells from CD patients and performing molecular profiling, we reinforced our previous *in vitro* and *in vivo* results and provided insight into the selection of target patients for IL23R-CAR-Treg therapy. Our data demonstrate IL23R-CAR-Treg as an effective potential treatment option for CD.

1. IL23R-specific activation towards cells isolated from CD patients was observed in IL23R-CAR-Tregs, suggesting:
   - The presence of IL23R in biopsy samples, predominantly in severe CD samples
   - Engagement and function of IL23R-CAR
2. Immunohistochemical analysis of intestinal biopsies from moderate-severe CD subjects confirmed that up to 60% of mucosal lamina propria cells express IL23R
3. Transcriptional profiling showed immune regulation and inflammation signatures were associated with IL23R-CAR-Treg efficacy
4. Multiple plasma biomarkers were identified to predict IL23R-CAR-Treg response and will be validated with a validation cohort



These results support further development of our IL23R-CAR-Tregs in clinical studies.

Acknowledgments

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