Original research

Biopsy and blood-based molecular biomarker of inflammation in IBD

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ABSTRACT

Objective IBD therapies and treatments are evolving to deeper levels of remission. Molecular measures of disease may augment current endpoints including the potential for less invasive assessments.

Design Transcriptome analysis on 712 endoscopically defined inflamed (Inf) and 1778 non-inflamed (Non-Inf) intestinal biopsies (n=498 Crohn's disease, n=421 UC and 243 controls) in the Mount Sinai Crohn's and Colitis Registry were used to identify genes differentially expressed between Inf and Non-Inf biopsies and to generate a molecular inflammation score (bMIS) via gene set variance analysis. A circulating MIS (cirMIS) score, reflecting intestinal molecular inflammation, was generated using blood transcriptome data. bMIS/cirMIS was validated as indicators of intestinal inflammation in four independent IBD cohorts.

Results bMIS/cirMIS was strongly associated with clinical, endoscopic and histological disease activity indices. Patients with the same histologic score of inflammation had variable bMIS scores, indicating that bMIS describes a deeper range of inflammation. In available clinical trial data sets, both scores were responsive to IBD treatment. Despite similar baseline endoscopic and histologic activity, UC patients with lower baseline bMIS levels were more likely treatment responders compared with those with higher levels. Finally, among patients with UC in endoscopic and histologic remission, those with lower bMIS levels were less likely to have a disease flare over time.

Conclusion Transcriptionally based scores provide an alternative objective and deeper quantification of intestinal inflammation, which could augment current clinical assessments used for disease monitoring and have potential for predicting therapeutic response and patients at higher risk of disease flares.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

⇒ Treatment targets in IBD are moving towards deeper levels of remission, from clinical to endoscopic and recently, histologic normalisations. How 'deep' should we go for long-term disease control is an on-going question.

WHAT THIS STUDY ADDS

⇒ Current measurements of IBD activity are likely under-representing persistent inflammation at the molecular level, which can be expressed using biopsy and blood scores.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our work lays a platform for augmenting current clinical practices associated with patient disease monitoring, stratification and therapeutic response management through the use of molecular scores of inflammation based on expression levels of specific genes measured in either mucosal biopsies or non-invasively, in circulating blood RNA.

INTRODUCTION

Inflammatory bowel disease (IBD) is a progressive inflammatory disease of the digestive tract characterised by periods of relapses and remission and consists of two types, namely, Crohn's disease (CD) and Ulcerative colitis (UC).[1 2] In the past two decades, the therapeutic goal in IBD has evolved from attaining mere symptomatic remission to achieving sustained clinical and endoscopic remission with the ultimate aim of disease modification defined as blocking natural progression to complications and surgery.[3–5] However, despite major advances in drug development and innovative therapeutic strategies, the proportion of patients in whom disease modification can be reached remains regrettably low.[6] More ambitious targets are now proposed such as combined endoscopic and histologic remission in UC and transmural healing in CD.[7–9] An approach already proposed in other immune-mediated disorders,[10–12] which has yet to be explored in IBD, is to target inflammation that

may silently persist at the molecular level even in macroscopically and/or histologically normal mucosa.

We, therefore, constructed a biopsy molecular inflammation score (bMIS) with the rationale that it may enable a more objective, granular and sensitive measure of disease activity applicable to patients with CD or UC. We also derived a circulating MIS (cirMIS) of gut inflammation using blood RNA transcriptomic data to develop a less invasive blood test of disease activity. Both bMIS and cirMIS were developed using the Mount Sinai Crohn's and Colitis Registry (MSCCR), a discovery cohort with ~1200 patients with IBD and controls.[13] As histological, endoscopic and clinical assessments were available on the same patient cohort, cross-comparisons of newly derived molecular with clinical scores could be performed. We then used seven different independent IBD data sets with available transcriptome data to evaluate the robustness of various aspects of the molecular score, including association with disease as well as association with treatment (compared with placebo) and treatment response, where clinical trial data were available.

METHODS

Discovery cohort: MSCCR

The MSCCR is a cross-sectional cohort consisting of patients with IBD and controls prospectively recruited during their endoscopy visit from December 2013 to September 2016.[13] Paired blood and biopsy RNA sequencing (RNA-seq) data were generated at the time of clinical, histological and endoscopic assessments (figure 1A). Institutional review board approval and informed consents were obtained. The Simple Endoscopic Score for Crohn's Disease (SESCD) was used[14] to classify CD endoscopic disease activity as inactive (0–2), mild (3–6), moderate (7–15) and severe (≥16).[15] The Mayo endoscopic measure was used to categorise UC as having: normal/inactive disease (0); mild disease (1); moderate disease (2) or severe disease (3).[16] Clinical disease activity measures included the Harvey-Bradshaw index (HBI) for CD and clinician-based Simple Clinical Colitis Activity Index (SCCAI) for UC. Clinically inactive disease was defined as an HBI <5 or an SCCAI <5 and active disease as an HBI >7 or SSCAI ≥5. Histological assessment was performed by a pathologist (A. Iuga) on biopsies taken adjacent to the specimen processed for RNA seq analysis. Control and patients with UC biopsies were scored for the Nancy index (0–4)[17] and Control and CD biopsies were scored according to the general histology activity score (GHAS) (0–13 using 7 of 8 scoring criteria, (does not include 'number of biopsy specimens affected')).[18–21] GHAS score and Nancy index are considered acceptable and reproducible methods for the histological scoring of disease activity in IBD. They provide an assessment of chronic and active mucosal inflammatory changes and gross epithelial damage.[22] Montreal disease phenotypic subclassifications including UC disease extent (E1, E2 or E3)[23] were available. Demographic information associated with this cohort is summarised in online supplemental table 1.

Validation cohorts

Cross-sectional cohorts

1.RTP. The road to prevention (RTP) cohort encompasses 346 subjects from 83 families with at least two first-degree relatives diagnosed with IBD and a set of unrelated healthy individuals with no family history of IBD or other chronic immune diseases and matched for age, gender and ethnicity. Among those, 32 participants were unrelated healthy individuals with no IBD family history, 179 were unaffected relatives of the 135 participants that were diagnosed with IBD, with an average age of 33 years old, both genders equally represented (49.1% men) and most participants as Caucasians (97.6%) and Ashkenazi Jewish (89.8%). Demographics associated with this cohort is summarised in online supplemental table 2.

2. The RISK (Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with Crohn's Disease) cohort[24] of treatment-naive paediatric CD (<17 years of age, 58.5% men) patients were studied using RNA-seq expression profiles from GSE57945, which included ileal biopsies from endoscopically defined inflamed samples (n=163), non-inflamed (n=55) and non-IBD controls (n=42) at the time of diagnosis. Demographics is summarised in online supplemental table 3 for RISK cohort.

Longitudinal cohorts

3) The CERTIFI GSE100833 series includes blood Affymetrix (HGU-U133 Plus) expression profiles from 226 antitumour necrosis factor alpha (anti-TNF) refractory patients with CD enrolled in the CERTIFI trial with ustekinumab (UST).[25] Clinical response at week 22 was defined as a decrease of 100 or more in Crohn's Disease Activity Index (CDAI) score from baseline at week 22.

4) The GEMINI-I/LTS and anti-TNF GSE73661 series includes colonic gene expression (Affymetrix, HGU-1.0 ST) profiles from 44 moderate-to-severe patients with UC enrolled in two vedolizumab (VDZ) efficacy trials (GEMINI-I/LTS).[26] Also included were 12 non-IBD and 23 UC colonic biopsies from patients before and 4–6 weeks after first infliximab (IFX) treatment. Response to therapy was defined as endoscopic mucosal healing (Mayo endoscopic score 0–1) and assessed at 6 weeks for VDZ and 4–6 weeks for IFX.

5) The UNIFI UC phase 3 clinical trial of UST involves moderate-to-severe patients with UC who had inadequate response to or unacceptable side effects from TNF antagonists, VDZ or conventional therapy.[27] An 8-week randomised induction trial and a 44-week randomised withdrawal maintenance trial was performed with primary endpoint of clinical remission (defined as a total score of ≤2 on the total Mayo scale and no subscore >1). Other endpoints included endoscopic improvement (defined as a Mayo endoscopic subscore of 0 or 1), histological healing (defined as neutrophil infiltration in <5% of crypts, no crypt destruction and no erosions, ulcerations or granulation tissue) and histoendoscopic mucosal healing (HEMH) requiring both endoscopic improvement and histological healing. Biopsy transcriptome data (microarray) were available from 550 patients, 358 genes of the 498 bMIS UC geneset were available to generate gene-set variation analysis (GSVA) scores. The data are available on GEO (GSE206285).

6) The UNITI-1/2 CD induction and maintenance trial for UST included primary/secondary non-responders to anti-TNF and patients in whom conventional therapy failed.[28] The primary endpoint for the induction trials was a clinical response at week 6 (defined as a decrease from baseline in the CDAI score of ≥100 points or a CDAI score <150). Of 623 patients with whole blood transcriptome available, 73 of the 103 genes in the cirMIS CD signature set were available to generate cirMIS scores. The data are available on GEO (GSE207465).

7) The UC ACT1 anti-TNF (IFX) adult cohort[29] with the biopsy microarray data set (GSE23597) consists of a cohort of patients who participated in the placebo-controlled Active Ulcerative Colitis Trial 1 (ACT1) study. Colonic biopsies were collected from a subset of randomised patients at protocol-specified time

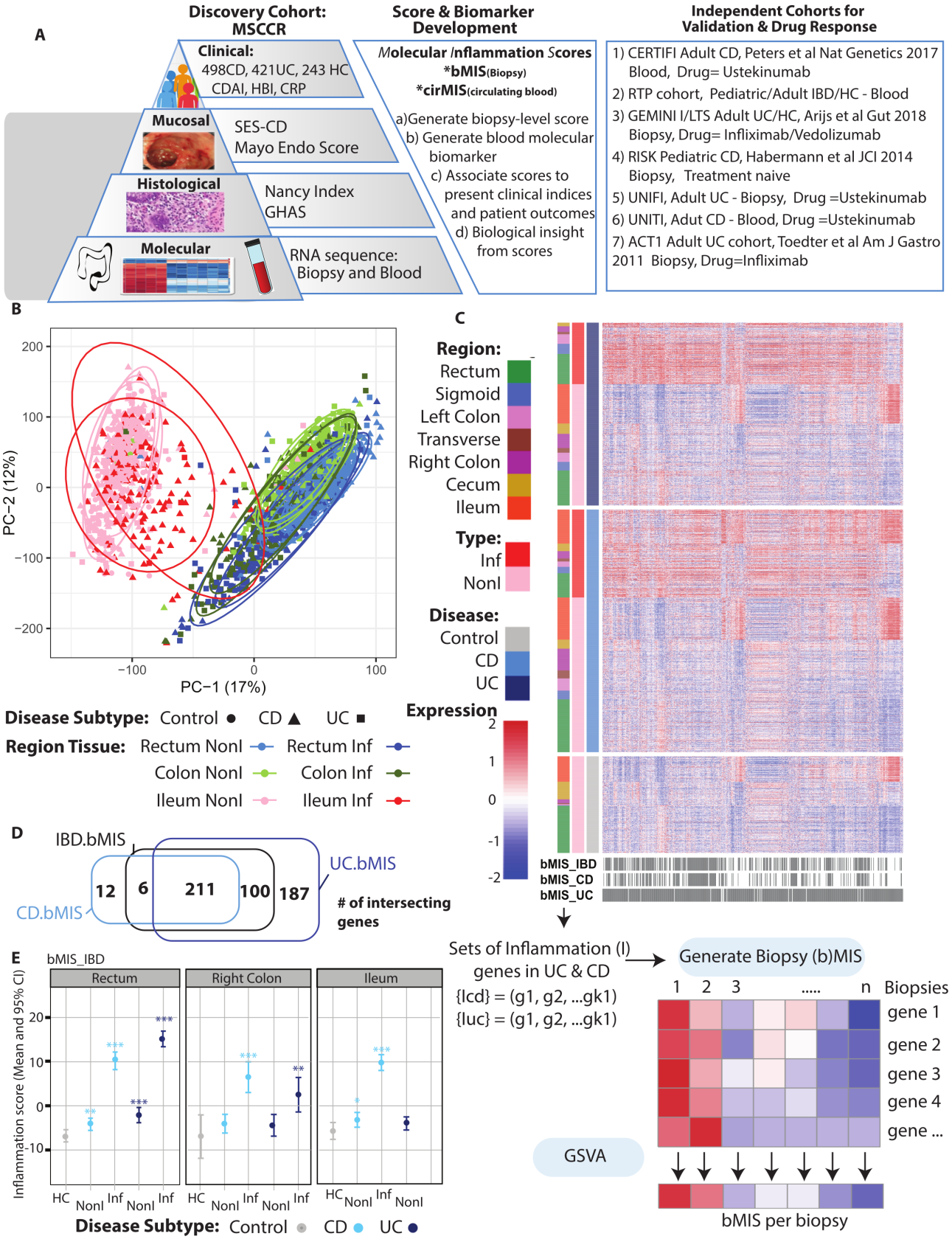


Figure 1 (A) Schematic of analysis plan. (B) Principal component analysis (PCA) of biopsy expression data for the Mount Sinai Crohn's and Colitis Registry (MSCCR) cohort by colonic region, endoscopically defined inflamed (Inf) or non-inflamed (NonI) tissue and by disease subtype (UC/CD and non-IBD controls). (C) Heatmap representing the expression levels of the set of bMIS genes (UC, CD or IBD) in IBD patient or non-IBD control biopsies and labelled by region, disease type and inflammation status. (D) Venn diagrams showing the number of differentially expressed genes for bMIS IBD, bMIS UC and bMIS CD signatures. (E) Estimated marginal mean (EMM) and 95% CI for bMIS_IBD levels by intestinal region, inflammation status and disease subtype (HC, healthy controls).

points after IFX therapy at 5 mg/kg or 10 mg/kg doses. Clinical response was defined as a decrease from baseline in the total Mayo score (of at least 3 points).

8) MSCCR patient subset follow-up. A subset of MSCCR patients with longitudinal follow-up were selected and all charts reviewed by an expert in IBD (RCU). This MSCCR subset included patients with UC and CD in endoscopic and histological remission at the time of the study. For UC, the criteria were Mayo endo score=0 and Nancy Score=0. For CD, SESCD=0 and GHAS score=0. Patients in remission were then categorised as having high or low cirMIS levels based on tertiles of expression (patients with UC, high n=8, low n=8 and patients with CD, high n=13, low n=13) and post-MSCCR study outcomes were investigated through chart review. The outcome was disease flare defined as a composite of any IBD-related hospitalisations, IBD-related surgery, need for new oral steroid and/or need for treatment escalation or new therapeutic agent due to active disease. We recorded date of the earliest adverse disease flare event or the date of the last follow-up if no event was seen.

MSCCR RNA-seq profiling

Biopsy and blood RNA from patients with MSCCR were extracted and processed in randomised batches as previously described[30] and in online supplemental methods. Coupled genotype data for the same patients were available.[31] For bMIS generation, biopsy data for patients with indeterminate IBD disease were removed (n=13) and biopsies identified as inflamed in the healthy control group were also removed (n=7). For subsequent analysis, biopsies from pouch patients (n=18 unique) were also removed. The data are available on GEO (GEO accession: GSE186507 for blood and GSE193677 for biopsy).

Generation of MISs, bMIS and peripheral blood (cirMIS)

Biopsy molecular inflammation score

Gene expression matrices from biopsy were generated from the count matrices using the voom transformation on the count matrix (see online supplemental methods). Voom-transformed gene expression data were modelled using a mixed-effect models with 'tissue type' (ie, endoscopically inflamed or non-inflamed), 'intestine biopsy region' (ileum, colon, rectum, etc) and 'disease subtype' (control, UC, CD) and its interactions as factors and a random factor for each patient, with technical (batch, RNA integrity number (RIN), ribosomal RNA (rRNA) rate, exonic rate) and relevant variables (age, gender and genetic principal components (PC) 1–5) as covariates. Notice that this model includes control samples of non-IBD subject, in order to account for the gut region effect not driven by inflammation; by including both region and disease as covariates in the development of bMIS. In this model, differences between endoscopically inflamed and non-inflamed tissue were assessed for each intestinal region (seven possible including: rectum, sigmoid, left colon, transverse, right colon, cecum and ileum) and disease subtype, thus defining intestinal region-specific and disease subtype-specific inflammation signatures (figure 1B,C). However, as we observed a strong correlation across the inflammation signatures, we generated a general IBD inflammation signature by fitting a model with tissue type, disease subtype and intestine biopsy region (no interactions) and an inflammation signature for each disease subtype by including only an interaction term for tissue type by disease subtype. From the IBD, or CD and UC subtype-specific inflammation gene signatures, we defined the markers of biopsy inflammation as genes differentially expressed (up-regulated genes only) between endoscopically inflamed and non-inflamed biopsies, at false discovery rate (FDR) <0.05 and fold change (FCH) >2 and the bMIS score was derived by using GSVA.[32] The inflammation score was built as the average z-score derived from the expression (adjusted for technical covariates) of the differentially expressed genes (DEGs) normalised by the square root of the number of genes.[33] As a result, each biopsy sample for the MSCCR cohort had a bMIS_IBD score as well as either a UC or CD disease subtype-specific score (bMIS_UC or bMIS_CD), depending on the patient's disease subtype diagnosis. This score is based on all DEGs, as we aimed to quantify the overall level of molecular inflammation (ie, a continuous score not an ordinal value like endoscopic or histological scores) that summarises the activity of all dysregulated genes, and not to develop a predictor of endoscopic inflammation status (yes vs no)). This rationale was also based on our experience in psoriasis where we developed a similar transcriptome scoring system (see online supplemental methods).

cirMIS

Blood gene expression data from 1030 patients for which intestinal biopsy transcriptome data were available, were used to identify genes whose expression in blood associated with the level of intestinal molecular inflammation. To obtain a patient-level, intestine molecular-based inflammation measure, we took advantage of the multiple regions sampled per individual and summarised the patient's individual bMIS scores into an intestinal-level (ileum-to-rectum) molecular inflammation score (iMIS) as described in online supplemental methods. The blood gene expression data were then modelled using a linear model with the continuous variables iMIS, technical covariates (RIN, batch, rRNA rate and exonic rate), imputed genetic PCs (#1–5), age at endoscopy, sex and IBD disease subtype. iMIS-associated blood genes were selected and used as the input to generate a circulating molecular score that reflects intestinal molecular inflammation (cirMIS) using GSVA.

As in the case of bMIS, in addition to cirMIS_IBD, we also generated subtype-specific cirMIS scores, that is, cirMIS_UC, cirMIS_CD) by identifying the blood gene signatures that were associated with the iMIS_CD and iMIS_UC in each CD and UC subcohorts, respectively.

Association of bMIS and cirMIS with IBD phenotypes, treatment effect and longitudinal outcomes

Statistical analysis was carried out using R language V.4.0.5[34] and its available packages. Each MIS for the discovery or validation cohorts was modelled using linear models after suitable preprocessing of the omics data with relevant factors depending on the comparison. When data were paired, that is, several biopsies were available for the same patient, or different time points, mixed-effect models were fitted including fixed factors and a random intercept for each subject using the *nlme* package in R. Model assessing changes with treatment included fixed effects for time, treatment, response and its interactions with time as fixed effects. For all models, classical model diagnosis was run. Marginal means, confidence intervals (CIs) were derived from fitted models using the *emmeans* package capabilities, and hypotheses of interests were tested using contrasts.

The tests described above are considered to be self-contained, where the association of genes other than the cirMIS/bMIS genes is not considered. As such, we also conducted 'competitive tests' where the null hypothesis assumes that genes in the bMIS/cirMIS are not more

associated with the phenotype than other genes. To this end, we performed bootstrap simulations, by evaluating the association with disease outcomes for 500 randomly selected gene sets of the same size as cirMIS/bMIS and quantified the 95% CI using the quantiles.

Logistic regression models were fitted using generalised linear models with the disease scores (either continuous or discretised as (low/high)) as factors and 10-fold cross-validation statistics were derived using the *boot* package. CIs were derived and DeLong's test was used to compare receiver operating characteristic curves between two sets of predictors.

Correlation of endoscopic, histological and clinical disease activity (continuous) measures with the molecular scores was assessed using Spearman correlations. Strength of said association is represented as a heatmap, where scores were clustered based on said distance using package *corr* using Ward agglomeration method.

Pathway enrichment analysis of bMIS and cirMIS-associated genesets

Gene sets were tested for enrichment using a Fisher's exact test with a Benjamini-Hochberg multiple test correction. The collection of genesets included BioPlanet pathways sourced from Enrichr[35]; CD and UC single-cell gene sets[36–38]; IBD GWAS candidate genes sourced from[39–41] and genes associated with IBD drug targets.[42] iRegulon,[43] within Cytoscape (V.3.9.0),[44] was used to detect enriched transcription factor motifs within the bMIS gene sets.

Patient and public involvement

Patients and/or the public were not involved in the design or reporting of this research.

RESULTS

A bMIS as a novel IBD activity measure

Figure 1A summarises the analysis plan and validation strategy with MSCCR cohort clinical characteristics in online supplemental table 1. Principal component analysis (figure 1B) of the intestinal transcriptomes revealed that region of biopsy was the largest factor contributing to variation in gene expression, followed by inflammation status, with disease subtype (UC vs CD) showing very little separation. To generate bMIS, we focused on genes found to be differentially expressed between endoscopically defined inflamed and uninflamed biopsies (figure 1C). Many genes were found commonly differentially expressed when inflammation was present in UC or CD, despite location of biopsy (figure 1D) and, therefore, bMIS scores were generated within disease type (bMIS_CD and bMIS_UC) or across disease subtypes (bMIS_IBD) (online supplemental table 4,5).

Using the sets of genes upregulated with inflammation, a GSVA score was generated, which compressed the expression of the inflammation gene set into a single value for each patient biopsy (figure 1C, online supplemental table 2). A summary of the bMIS scores according to disease type and region sampled is shown (figure 1E). In general, the bMIS of endoscopic-defined inflamed biopsies was 10-fold higher than those of non-inflamed biopsies. Notably, bMIS scores were significantly higher in non-inflamed biopsies relative to non-IBD control biopsies. Overall, these data suggest that molecular scores have higher sensitivity of detecting disease activity than macroscopic assessment on endoscopy and can also distinguish disease from non-disease state.

bMIS strongly correlates with current clinical disease activity measures

The bMIS score was based on qualitative information of inflammation (ie, binary status of 'inflamed' or 'non-inflamed'), and as such it was important to examine if the intestinal-based molecular scores capture disease activity metrics. To assess whether intestinal-based molecular scores segregate with disease, we associated bMIS scores to clinical, endoscopic and histological definitions of disease activity. A significantly higher bMIS for both CD and UC biopsies was observed in patients with active versus inactive disease as defined by HBI or SCCAI clinical disease activity scores (figure 2A, upper and lower panels). We observed a positive correlation between bMIS scores and endoscopic assessments of severity, with the most severely affected biopsies, according to the SESCD or Mayo endo scores, having the highest bMIS values (figure 2B). A significant number of biopsies, sampled nearby the biopsy taken for RNA sequencing analysis, were also evaluated histologically. A significant positive association was also observed between the GHAS and Nancy index histological pathological scores and the bMIS scores in control, IBD non-inflamed and inflamed biopsies. We also noted that bMIS could identify molecular inflammation where the histological scores were normal (score of 0 or 1) supporting the value of increased granularity with molecular information compared with the 13-factor or 4-factor range of the GHAS or Nancy index, respectively (figure 2C). Overall, we noted that the highest correlations were seen between bMIS values and any other clinical measure of IBD disease (figure 2D). Furthermore, the bMIS values of UC and CD were highly correlated, suggesting that disease-specific scores have little added value.

Generation of a circulating blood biomarker of bMIS: cirMIS

We next sought to develop a less invasive circulating molecular biomarker. To do this, we took advantage of the multiple regions sampled per individual and summarised the patient's individual bMIS scores into an iMIS (figure 2E). We first verified that iMIS levels, like bMIS levels, also significantly associated with clinical, endoscopic and histological definitions of disease activity (data not shown). We next demonstrated that iMIS levels were significantly and positively correlated with the known IBD activity biomarkers, namely, blood CRP or faecal calprotectin levels (figure 2F). These observations supported generating an equivalent patient-level molecular blood biomarker. To do this, we derived a set of genes that associated with iMIS and created a patient-level blood-based MIS (cirMIS), using GSVA (online supplemental table 4,5).

Similar to bMIS and iMIS, we show that cirMIS levels could distinguish cases from controls as well as significantly and positively associated with clinical, endoscopic or histologic assessments of intestinal inflammation (figure 3A–D). A significant overlap of the cirMIS geneset with the bMIS geneset was observed in addition to a strong correlation between cirMIS and iMIS levels (figure 3E and online supplemental figure 1A).

As an additional check, we also performed competitive tests to examine whether the genes involved in the generation cirMIS/bMIS are significantly associated with disease outcomes as compared with random geneset selections (see the Methods section). In all cases, we confirmed that the associations of

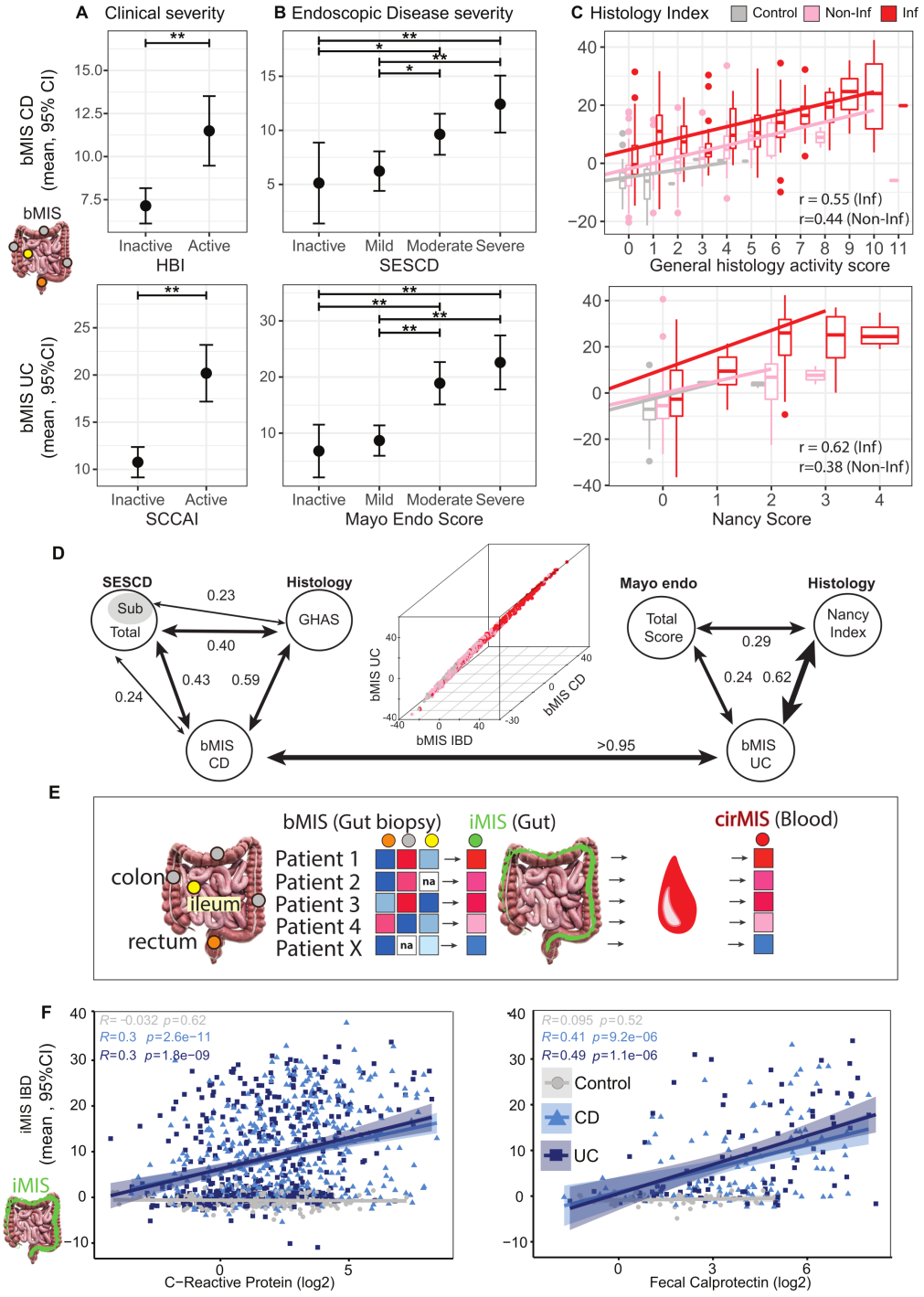


Figure 2 Association of bMIS in inflamed tissue with (A) clinical (HBI for CD patients, SCCAI for UC patients), (B) endoscopic (SESCD for CD patients, Mayo score for UC patients) and (C) histological (GHAS for CD patients, Nancy score for UC patients) disease severity for CD (top) and UC (bottom). (A, B) Estimated marginal mean (EMM) and 95% CI for bMIS estimated from a mixed-effect model including clinical activity or disease severity, age, sex and region as fixed-effects. (C) Scatter plots representing the distribution of bMIS across histological scores for CD and UC with corresponding regression line. The pink and red lines correspond to the regression line for inflamed and non-inflamed tissue. (GHAS (top): Inflamed tissue, bMIS=2.619 + 1.997*GHAS, Pearson r: 0.55; Non-inflamed tissue, bMIS=−3.926+2.018*GHAS, Pearson r: 0.44; Nancy (bottom): Inflamed tissue: bMIS=1.74+8.457*Nancy, Pearson r: 0.62; Non-inflamed tissue: bMIS=−5.269+5.216*Nancy, Pearson r: 0.38). *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. (D) Pair-wise correlation analysis (Spearman values) between bMIS scores in biopsies and corresponding endoscopic and histologic scores for CD (left) or UC (right) in MSCCR patients. 3D plot showing correlations (Spearman) between bMIS_UC, bMIS_CD and bMIS_IBD. (E) Schema showing the process of obtaining an intestine-level molecular-based inflammation measure (iMIS) per patient using the multiple regions sampled per patient and their bMIS (biopsy-based) scores (see methods). The blood gene expression data were then modelled using a linear model with the continuous variable iMIS (see methods) to identify genes that reflect intestinal inflammation and then generate a circulating molecular score (cirMIS) using GSVA. (F) Scatterplots between iMIS_IBD levels and CRP (log2) (left) or faecal calprotectin (log2) (right) within each group (Control, CD and UC), with Pearson's correlation coefficients r and p value. bMIS, biopsy molecular inflammation score; CD, Crohn's disease; GHAS, general histology activity score; HBI, Harvey-Bradshaw index; MSCCR, Mount Sinai Crohn's and Colitis Registry; SCCAI, Simple Clinical Colitis Activity Index.

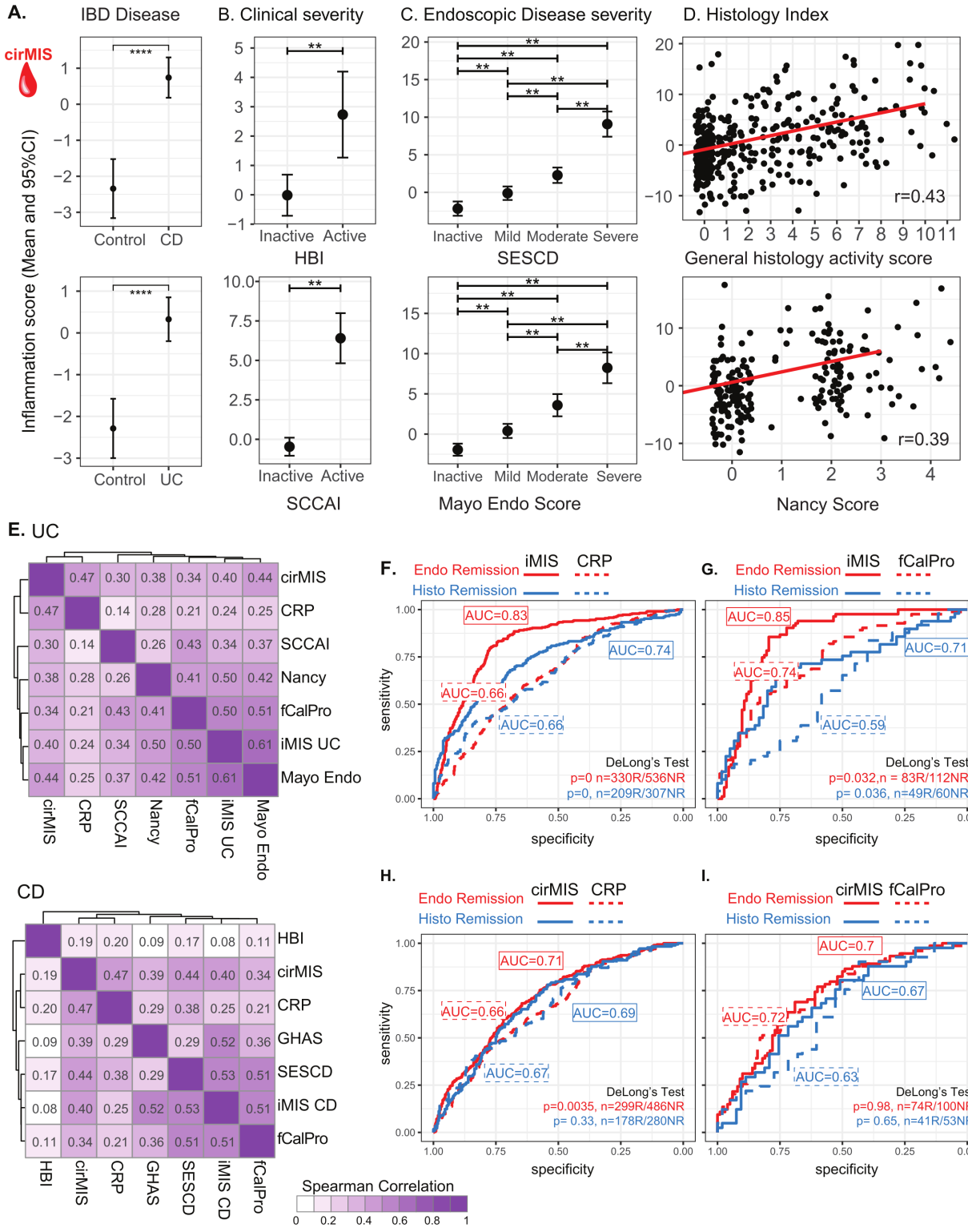


Figure 3 Association of cirMIS with IBD Disease (A) as well as clinical (B) endoscopic (C) and histological assessments (D). (A–C) Estimated marginal mean (EMM) and 95% CI for cirMIS from a mixed-effect model including IBD disease status (A) or clinical disease severity (B) or endoscopic disease severity (C), age, sex and genetic PCs as a fixed-effects. (D) Scatter plots representing the distribution of cirMIS across histological scores, maximum GHAS for CD and maximum Nancy score for UC. The red line corresponds to the regression line (Max GHAS (top): cirMIS=−1.756+0.901*GHAS, Pearson r: 0.43; Max Nancy (bottom) cirMIS=−1.21+1.802*Nancy, Pearson r: 0.39). (E) Heatmaps showing the Spearman correlations between iMIS and cirMIS with molecular (CRP and faecal calprotectin (fCalPro)), endoscopic (HBI for CD and Mayo for UC), histological (GHAS for CD and Nancy for UC), and clinical markers (SESCD for CD and SCCAI for UC) of UC (upper) and CD (lower). (F–I) Comparison of iMIS and cirMIS with CRP and faecal calprotectin (fCalPro) to classify endoscopic remission (SESCD<3 in CD patients or Mayo endo score=0 in UC patients) and histological remission (GHAS score=0 in CD patients or Nancy score=0 in UC patients). (F–I) Delong's method was used to compare AUCs. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. CirMIS, circulating molecular inflammation score; CRP, C reactive protein; GHAS, general histology activity score; HBI, Harvey-Bradshaw index; SCCAI, Simple Clinical Colitis Activity Index.

cirMIS/bMIS scores with IBD activity measures were significantly higher and far removed from the 95% CI of scores generated using randomly selected genesets (online supplemental figure 1B and data not shown).

Comparison of b/iMIS and cirMIS to current biomarkers of activity

A summary of the cocorrelations among the various molecular scores of inflammation, existing biomarkers and clinical, endoscopic and histological disease activity assessments are shown in figure 3E (online supplemental table 6). Overall, this analysis supports that scores based on gene expression levels are reflective of active intestinal inflammation. We next used logistic regression to compare the intestinal-level bMIS (ie, iMIS) and cirMIS scores with CRP and faecal calprotectin in their ability to identify patients in (1) endoscopic remission (SESCD <3 in patients with CD or Mayo endo Score=0 in patients with UC) or (2) histological remission (GHAS score=0 in patients with CD or Nancy score=0 in patients with UC). The results are summarised in figure 3F–I. In the MSCCR cohort, iMIS out performed both CRP and faecal calprotectin. CirMIS was significantly better at identifying patients in endoscopic remission as compared with CRP and equivalent to faecal calprotectin. Overall, adding CRP or faecal calprotectin to models with either bMIS/cirMIS scores did not lead to gains in AUC. However, performance prediction, in general, was better, in models where bMIS/cirMIS scores were used, as compared with models with CRP of faecal calprotectin levels alone (online supplemental table 7). Consistent with the logistic regression analysis, we also observed that cirMIS was in general more strongly correlated with endoscopic, histological and clinical scores than CRP supporting that cirMIS could be a better blood biomarker than CRP (figure 3E). With respect to faecal calprotectin, in general, comparable correlations were observed compared with the molecular scores, supporting that these new scores are potential alternatives (or supplementary) to faecal calprotectin.

Replication of bMIS/cirMIS in independent IBD cohorts

We curated transcriptome datasets (blood or biopsy) from four separate IBD cohorts and using the same gene sets as in the discovery MSCCR cohort, we generated MISs and associated them with available clinical information. Figure 4A shows significantly higher bMIS levels in biopsies taken at week 0 from adult patients with UC during two phase 3 trials of VDZ (GEMINI I and LTS) or prior to IFX therapy,[13] as compared with non-IBD control individuals. Furthermore, a significantly higher bMIS was observed in the patients with UC with Mayo endo score of 3 versus 2, supporting that bMIS levels also correlate with levels of disease activity. Figure 4B shows that bMIS levels were significantly higher in the inflamed ileal biopsies as compared with the non-inflamed ileal biopsies from the RISK CD paediatric, treatment-naïve cohort, supporting utility of this score in younger patients with IBD and its utility in newly diagnosed cases. bMIS levels were also significantly higher in non-involved biopsies compared with non-IBD RISK cohort controls, again underlying that molecular disease exists where visual endoscopic assessments are normal.

The third IBD cohort consisted of anti-TNF non-responsive adult patients with CD as part of CERTIFI UST clinical trial. We confirmed a positive and significant correlation of cirMIS levels to faecal calprotectin or CRP levels (figure 4C). The CDAI scores, however, were poorly associated with cirMIS levels. Blood transcriptome data were available from a recently generated cohort of Orthodox Jewish paediatric and adult patients with IBD as part of an RTP MSSM-Janssen initiative. cirMIS levels were found significantly higher in patients with UC or CD relative to healthy controls (figure 4D). We also noted that the more generalised bMIS_IBD or cirMIS_IBD scores, which performed similarly in MSCCR to disease-specific scores, also replicated outside of the discovery MSSCR cohort (data not shown and online supplemental figure 2A–C). Overall, these four independent IBD cohorts and the discovery MSCCR cohort support that the genes and their summation into a molecular score are reproducibly showing association to disease activity and severity and in some cases, revealing disease where endoscopy evaluations appear normal.

With respect to UC disease extent, we also observed that bMIS levels were significantly higher in E3/E2 MSCCR patients than those with limited E1 disease (online supplemental figure 2D). This likely reflects the higher burden of inflammation in E2/3 versus E1. This IBD subphenotype association was also distinguished by cirMIS (online supplemental figure 2D), an observation independently replicated as cirMIS_UC levels were also higher in E3 patients with UC compared with those with limited disease in the RTP IBD cohort (online supplemental figure 2E).

Molecular inflammation measures and IBD therapies

To evaluate if cirMIS or bMIS scores change in response to therapy and drug response, we subset CD or UC MSCCR patients according to anti-TNF responders and non-responders based on current anti-TNF self-reported use and an SESCD<5 or Mayo endo score <2. We observed that both bMIS and cirMIS levels were higher in the inflamed biopsies of anti-TNF non-responders as compared with responders (online supplemental figure 2F). As MSCCR is a cross-sectional cohort, we next evaluated our molecular scores in independent cohorts including existing clinical trial data sets.

bMIS and UC

bMIS scores were evaluated in the UC biopsies from GEMINI-I/LTS trial participants following VDZ (or placebo) treatment as well as in patients with UC after IFX therapy.[26] No significant differences were observed in bMIS levels between baseline and week 6 biopsies in the placebo group; however, significant decreases were observed in the IFX or VDZ treated groups (figure 5A). When patients were stratified by response to drug (defined as endoscopic healing), a statistically significant decrease in bMIS was observed in the IFX or VDZ responder group as compared with non-responder groups (figure 5B). A significant positive correlation was observed between the change in bMIS score and change in Mayo endo score across all the patients, supporting that molecular descriptors of inflammation do reflect other metrics of disease activity (figure 5C).

In the ACT1 cohort of IFX treated adult patients with UC[29] the change in baseline bMIS levels compared with either week 8 or week 30 bMIS levels, was greater in IFX-treated patients as compared with the change observed in the placebo group. When patients with IFX were stratified by response to drug (defined as the clinical response at week 30) a greater decrease in bMIS, as compared with baseline, was observed in responders as compared with that observed in non-responders. In the UNIFI UC cohort on UST therapy, a similar pattern was observed, with bMIS levels showing the greater delta compared with baseline in treated patients (as compared with placebo) as well as in responders (defined as clinical remission) compared with non-responders (figure 5F). Similar results were observed when using

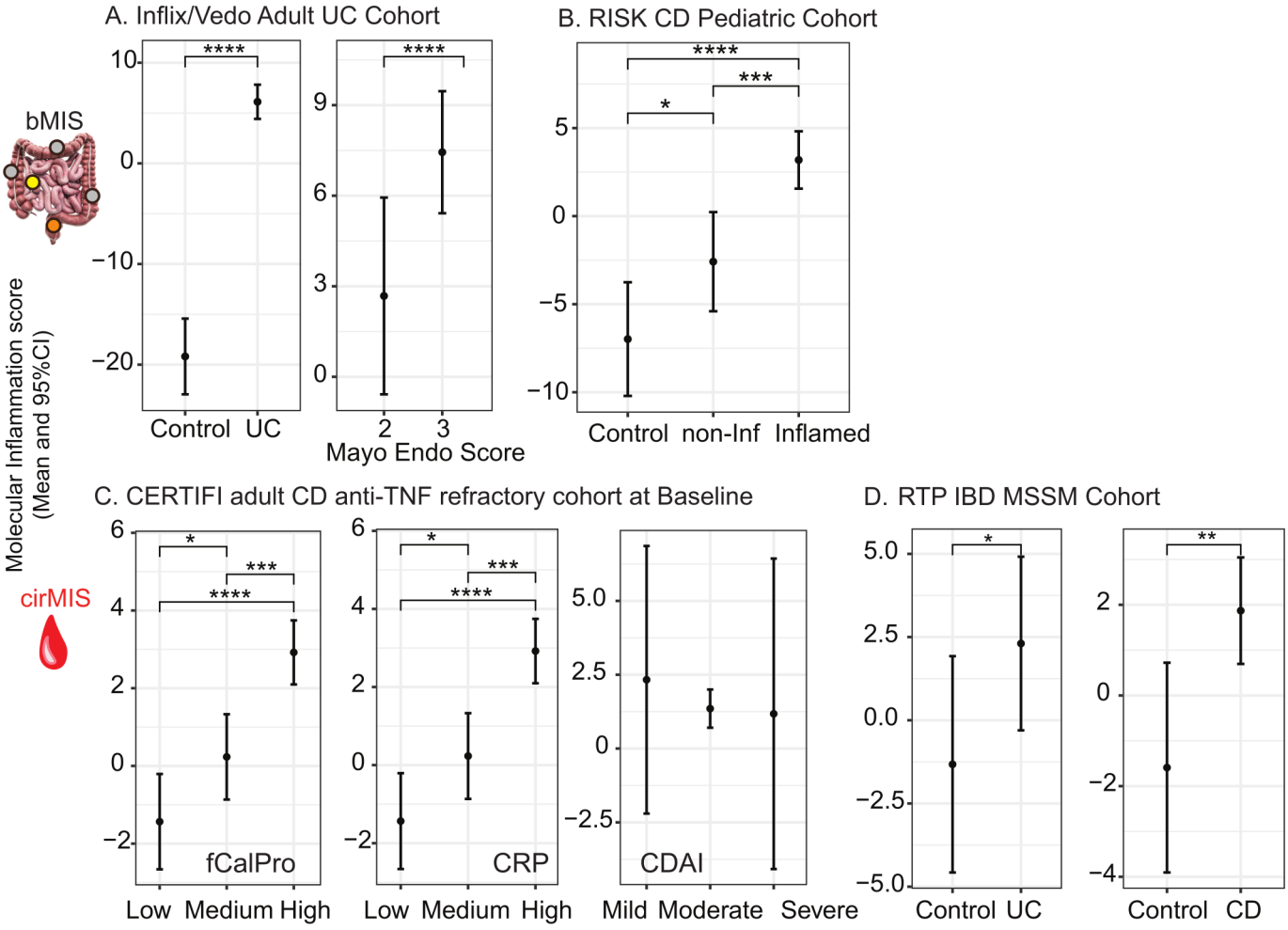


Figure 4 Validation of bMIS/cirMIS in independent IBD cohorts. (A) Line plots showing bMIS_UC can differentiate between control and UC disease status and associate with Mayo endoscopic scores in colonic biopsies from GEMINI-I/LTS trial and anti-TNF UC participants. (B) Line plots showing bMIS_CD could differentiate between control, non-inflamed ileum and inflamed ileum biopsies form the RISK paediatric CD Cohort. (C) Line plots showing cirMIS_CD associates with faecal calprotectin (fCalPro) and CRP, but not CDAI in the CERTIFI CD cohort. (D) Line plots showing cirMIS_UC and cirMIS_CD can differentiate between control and UC or CD disease status in the RTP-IBD MSSM cohort. (A–D) Each plot represents the estimated marginal mean and 95% CI through a linear mixed-effect model on the baseline data. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. bMIS, biopsy molecular inflammation score; CDAI, Crohn's Disease Activity Index; CirMIS, circulating MIS; CRP, C reactive protein; RTP, road to prevention; TNF, tumour necrosis factor.

the IBD-centric molecular scoring system (data not shown). Overall these data highlight the potential utility of bMIS as a valuable measure of disease activity in patients with UC distinguishing placebo and non-response states.

cirMIS and CD

In the CERTIFI CD trial, cirMIS levels were significantly lower in UST-treated patients at week 4 and 6 compared with their baseline levels, with no effects in the placebo group (figure 5G). Change in cirMIS levels was also dose-dependent (figure 5H). There was an induction and maintenance phase with response at week 22 defined as a decrease of 100 or more in CDAI score from baseline. We observed that week 6 UST-treated patients that continued to receive UST in the maintenance phase had a significantly lower cirMIS level compared with patients that were switched to the placebo group in the maintenance phase. Furthermore, we noted that UST-treated patients that were considered week 22 responders also had lower cirMIS levels compared with non-responders (figure 5G–H).

In the UNITI CD trial cirMIS levels were significantly lower, already at week six compared with their baseline levels, a decrease significantly higher in UST-treated patients compared with placebo. UST-treated patients that were considered week 8 responders (defined as clinical remission) demonstrated a greater reduction in cirMIS levels at week 6 or 8 (as compared with their baseline) when compared with the changes observed in non-responders (figure 5I). Furthermore, in comparison to the blood biomarker CRP, the effect size of the treatment effect between responders from non-responders was almost double when cirMIS levels were used, potentially supporting a stronger clinical trial utility with respect to required sample sizes, as compared with CRP (figure 5M).

Relationship between molecular inflammation and long-term outcomes

Having demonstrated that molecular inflammation is associated with current indicators of IBD disease activity and tracks with anti-IBD therapy and response to therapy, we then looked at the

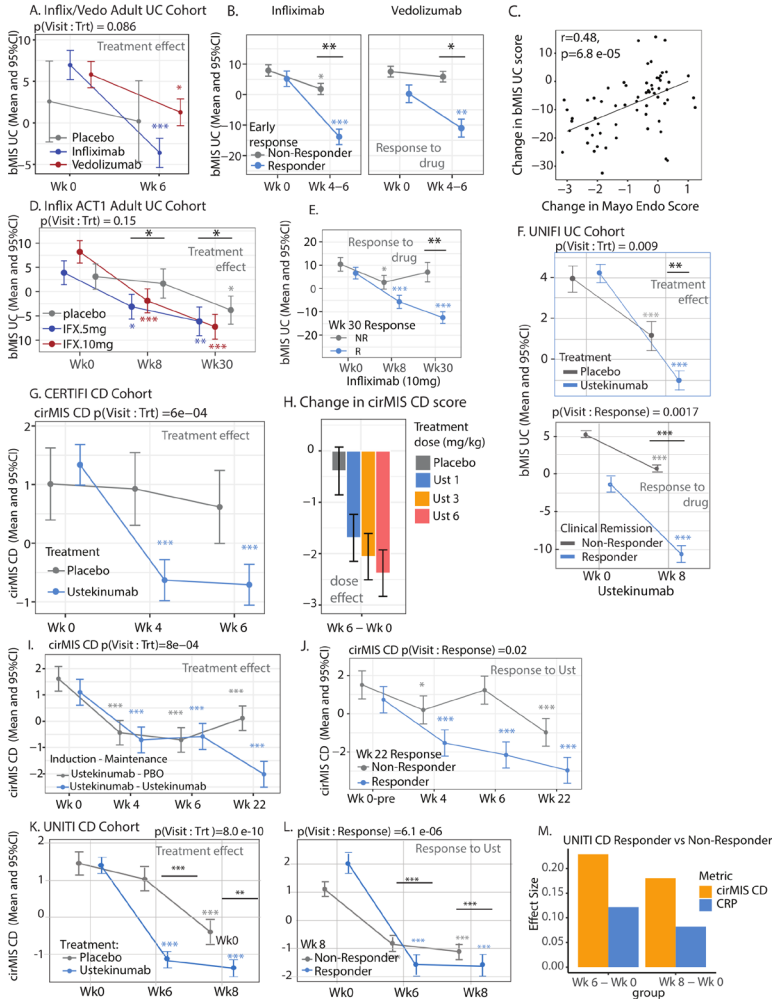


Figure 5 Molecular scores of inflammation and IBD treatment effects and response. (A) Change in bMIS_UC levels from week 0 to week 6 in placebo, IFX and VDZ groups in colonic biopsies from GEMINI-I/LTS trial and anti-TNF UC participants. The linear mixed-effect model included visit, treatment and, its interaction as fixed effects, and random intercept for each subject. (B) Change in bMIS_UC levels from week 0 to week 4–6 in responders and non-responders within the IFX and VDZ medication groups in colonic biopsies. Patients with induction treatment as infliximab or VDZ were analysed. Linear mixed-effect model with a three-way interaction visit, treatment and week 6 or week 4–6 status as fixed effects, and random intercept for each subject. Response to therapy was defined as endoscopic mucosal healing (Mayo endo score 0–1). (C) Correlation between changes in Mayo endo scores and bMIS_UC scores between week 0 to week 4–6, week 6 or week 52 in GEMINI-I/LTS trial and anti-TNF UC participants. (D) bMIS_UC scores in colonic biopsies from ACT1 UC trial participants at various timepoints treated with either 5 or 10 mg/kg of IFX. The linear mixed-effect model included visit, treatment and, its interaction as fixed effects, and random intercept for each subject. (E) bMIS_UC levels from week 0 to week 4 and 30 in week 30 responders and non-responders within the infliximab 10 mg/kg groups in colonic biopsies from ACT1 UC trial participants. Patients with induction treatment as infliximab and maintenance were studied. Linear mixed-effect model was used. Clinical response was defined as a decrease from baseline in the total Mayo score (of at least three points). (F) bMIS_UC levels from week 0 to week 8 in placebo and UST groups in colonic biopsies from UNIFI UC trial participants (upper panel). The linear mixed-effect model included visit, treatment and, its interaction as fixed effects, and random intercept for each subject. Change in bMIS_UC levels from week 0 to week 8 in responders and non-responders within the UST medication groups in colonic biopsies from UNIFI trial participants (lower panel). Patients with induction treatment as UST were analysed. Response to therapy was clinical remission (defined as a total score of≤2 on the Mayo scale and no subscore>1). (G, H) Changes in cirMIS_CD levels during the induction phase (from week 0 to 6) differentiate placebo from UST treated CERTIFI CD patients and (G) exhibit a dose response to UST (1, 3 and 6 mg/kg) (H, I) Changes in cirMIS_CD levels at weeks 0, 4 and 6 (induction phase) and during maintenance treatment starting week 6 in CERTIFI CD patients with UST induction treatment and UST or placebo as maintenance treatment. Plots represent the EMM±SEM estimated through a linear mixed-effect model with visit, treatment combination and, its interaction as fixed effects, and random intercept for each subject. (J) cirMIS_CD levels at week 0, 4, 6 and 22 in week 22 responders and non-responders in CERTIFI CD patients that received UST during induction and maintenance phases. EMM and±SEM from a linear mixed-effect model with visit, response status at week 22 and its interaction as fixed effects, and random intercept for each subject. Clinical response was defined as a decrease of 100 or more in CDAI score from baseline at week 22. (K) Changes in cirMIS_CD levels during the induction phase (from week 0 to 8) differentiate placebo from UST treated UNITI CD patients as well as responders compared with non-responders. (L) Response (at week 8) was defined as clinical remission (CDAI score<150 points). (M) Effect size of the delta between either week 6 and baseline or week 8 and baseline for either cirMIS_CD or CRP levels as determined in responders versus non-responders. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. Black line/asterisk represents significance of the delta between a timepoint and baseline for treated versus placebo group. bMIS, biopsy molecular inflammation score; CRP, C reactive protein; cirMIS, circulating MIS; EMM, estimated marginal mean; IFX, infliximab; SEM, standard error of the mean; TNF, tumour necrosis factor; UST, ustekinumab; VDZ, vedolizumab.

impact of molecular inflammation on short-term and long-term IBD outcomes beyond current disease activity metrics. We tested this hypothesis in two clinically relevant scenarios.

The first scenario included asking if differing levels of baseline bMIS or cirMIS levels of patients deemed to have similarly active disease macroscopically (eg, same endoscopy scores) have different short-term responses to therapy. We first addressed this question using the GEMINI-I/LTS and IFX UC cohort data. We initially observed that the baseline bMIS values were significantly associated with Mayo endo score (p=0.016), confirming the association seen in MSCCR. We then observed that baseline bMIS was significantly higher (3.08 vs 7.5, d=4.44, p=0.023 figure 6A) in patients who were considered endoscopically responders at week 6. This difference was more marked in patients with baseline Mayo endo score of 2 (4.56 vs −0.24, d=4.89), than Mayo endo score of 3 (4.98 vs 8.5, d=3.52, p=0.052). We then separated the patients in low bMIS (n=27) and high bMIS (n=27) groups using as cut-off the median (4.519) baseline bMIS value for all randomised patients. Rates of drug non-response were similar among patients with Mayo endo score 2 than Mayo endo score 3 (64%, vs 75%, OR=1.6, p=0.45). On the other hand, patients with high bMIS were significantly more likely (OR=3.92,p=0.045) to be non-responders to IFX/Vedo than patients with low bMIS (85% vs 58% response rates, respectively, figure 6A—right panel), and this difference was higher in patients with Mayo endo score=2 (data not shown), indicating that there is clinical relevance to molecular inflammation, beyond that assessed by endoscopy scores. These results were confirmed in additive logistic model where only bMIS but not Mayo endo score was associated with response at week 6 (p (Mayo endo score)=0.87, p (bMIS)=0.058). We next aimed to validate this observation in a phase 3 clinical trial by repurposing the UNIFI UC cohort. We first noted that patients who were non-responders at week 8 (clinical remission) had higher levels of baseline bMIS_IBD than responders (p=2.2e-08, online supplemental figure 3A). Given the many response definitions possible in this clinical trial, we next determined the likelihood of being a treatment responder according to various week 8 definitions, spanning from clinical remission, endoscopic improvement, histological healing and HEMH. A general finding across all response variables was that the OR was significantly greater for patients having lower baseline bMIS levels compared with higher bMIS levels. Importantly, the OR remained substantially higher when both bMIS and total Mayo score were considered in an additive model, thus strengthening the argument that molecular inflammation captures disease relevant signal not observed with standard endoscopic scores (figure 6B). As this suggests that bMIS has better prognostic value compared with currently used parameters, we compared the prognostic value of bMIS_UC to predict endoscopic healing at week 8 in the UNITI UC trial. An optimal cut point was first determined for baseline bMIS levels by maximising the sum of sensitivity and specificity and then a logistic regression analysis was run. Indeed, baseline bMIS levels performed significantly better than Mayo score, with AUC's of 0.73 and 0.67, respectively (p=0.05 by DeLong's test).

The second clinically relevant scenario we evaluated was whether patients in endoscopic and histological remission, yet with high residual molecular inflammation, have poorer long-term outcomes. We first addressed this question, by subsetting MSCCR patients with UC and CD, according to those who were in endoscopic and histological remission at the time of the study. Thirteen UC (seven=high and six=low cirMIS levels) and 19 CD (10=high and 9=low cirMIS levels) patients had follow-up data and were included in this analysis. In general, higher iMIS and cirMIS levels at the time of MSCCR endoscopy were observed in patients with IBD that subsequently had a disease flare to those that did not (figure 6C), while CRP levels were equivalent (p=0.55, data not shown). Despite the sample size, patients with UC with low cirMIS levels had lower rates of disease worsening events (16%) compared with those with higher cirMIS level (57%, p=0.15) at the time of the MSCCR study (figure 6D). For CD, the rate of events reflecting disease worsening was the same between patients in the high and low cirMIS groups (online supplemental figure 3), but survival analysis shows that events occur earlier in those with higher bMIS (figure 6E). Specifically, the Cox models reported a HR 2.3 times higher for high than low cirMIS patients ((HR for IBD=2.3, CI (0.66 to 7.82) (p=0.19), with HR=3.1 (0.31, 30) (p=0.3) for UC and 1.71 (0.34 to 8.58) (p=0.5) for patients with CD). Given the small sample size in MSCCR with longitudinal follow-up, we next assessed the consequence of residual molecular inflammation on potential disease relapse in the UNITI UC clinical trial cohort. We subset the UNITI UC cohort according to patients considered responders at week 8 by the various macroscopic and microscopic assessments (clinical remission (n=33), endoscopic improvement (n=44), histological healing (n=65) and combined HEMH (n=34)), into high and low bMIS expressing groups, based on their median value at week 8. We then assessed the proportion of patients in each group that were in clinical remission at week 44. Figure 6F summarises the estimated OR of clinical remission in the high versus low bMIS groups and compared with their week 8 Mayo score. In general, given any week 8 response definition, those patients with higher residual molecular inflammation at week 8, were generally less likely to maintain clinical remission status at week 44 compared with the low molecular inflammation group. For example, in week 8, HEMH responders, those patients with lower bMIS levels at week 8, were 5 times more likely to maintain clinical remission status at week 44, then those HEMH responders at week 8, that had higher week 8 bMIS levels (p=0.037). The OR for the Mayo scores was non-significant and compared with bMIS, always lower, with the OR based on bMIS ranging from 1.5 to 6.15 times higher than the OR based on Mayo scores, depending on the week 8 response definition.

With respect to CD, while we could assess the association of cirMIS levels with treatment effect and response based on clinical remission (figure 5K–M) within the UNITI trial, the number of drop outs for longitudinal analysis associated with endoscopic or histological outcomes was very high and as such we were underpowered to assess whether high residual cir/bMIS levels in week8 UNITI CD responders was associated with relapse.

bMIS provides a biological perspective of a disease activity measure

Our MISs, based on expression of a known set of genes, compared with other clinical metrics, directly inform on activity of biological processes underlying the observed phenotype. A summary of the genes according to recurrence in the bMIS/cirMIS gene panels reveals several top recurring genes that have been previously associated with IBD, such as biomarkers of unstable disease control (CXCL8(IL-8), S100A8)[45] or potential failure of anti-TNF therapy (Oncostatin M/*OSM*[46] (figure 7A, online supplemental table 5). BioPlanet pathways enriched in the bMIS_IBD genesets, included 'Oncostatin M', 'TNFa effects on cytokine activity, cell motility and apoptosis' and 'Interleukin-23, IL-17 and Jak-STAT signalling pathways are supportive of known anti-IBD targets and potentially a source of novel

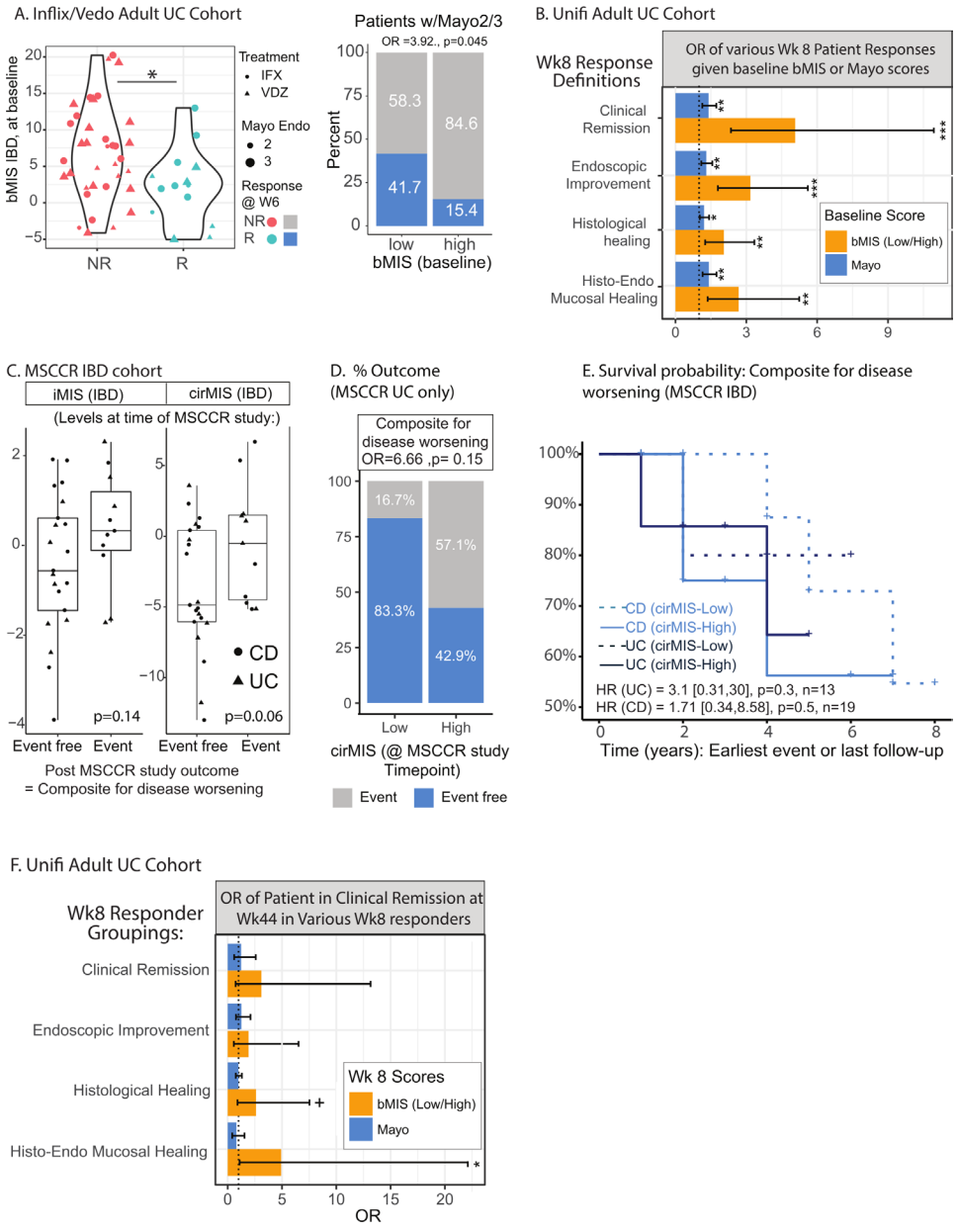
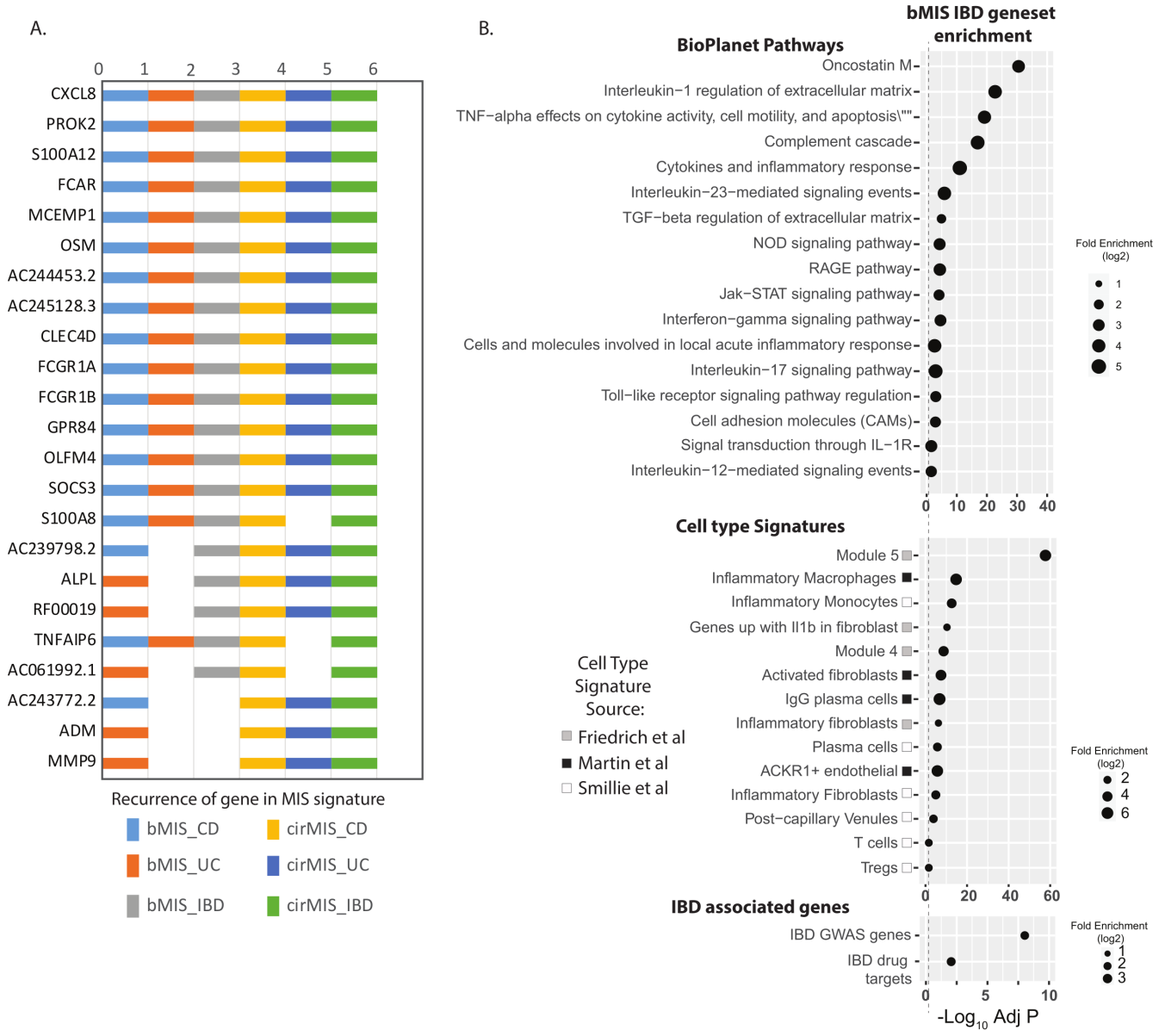


Figure 6 Residual molecular inflammation and clinical outcomes. (A) Baseline bMIS_IBD levels in patients from the GEMINI-I/LTS UC and anti-TNF treated patients according to response to therapy (endoscopic mucosal healing (Mayo endoscopic score 0–1) as assessed for VDZ at W6 and for IFX at W4–6) (left panel). The proportion of patients that were considered responders to therapy in the baseline high versus low bMIS group (right panel). (B) UNIFI adult UC cohort and the odd's ratio of having a response, according to various definitions from clinical remission, endoscopic improvement, histological healing and combined histo-endoscopic mucosal healing (see methods) at week 8 of therapy (UST) in baseline high versus low cirMIS group's (based on median levels) or according to baseline Total Mayo score. An additive model was used. (C) A subset of MSCCR patients were identified that were in endoscopic and histological remission at the time of the MSCCR study (called MSCCR endo-histo-remission) see methods). These patients were then categorised as having high or low cirMIS levels based on the tertiles of expression and their post-MSCCR outcomes considered as a composite score to reflect disease worsening were reviewed in their charts. The levels of bMIS_IBD and cirMIS_IBD of the MSCCR endo-histo-remission patient subsets were generally higher in those patients that subsequently had a disease worsening event post-MSCCR study. For the composite score of events reflecting disease worsening, one of six MSCCR UC patients with low cirMIS had an event while four out of seven patients with a high cirMIS had an event (D). For the CD MSCCR endo-histo-remission patients, the proportion of patients that were event free was the same between high and low cirMIS levels (online supplemental figure 3B). However, Kaplan-Maier survival curves (E) show that patients with higher cirMIS levels, were more likely to have a disease worsening event recorded earlier than compared with the low cirMIS group (in either UC or CD subsets). Specifically, the Cox models reported that for UC patients, the HR is 3.1 times higher [0.31,30, p=0.3] than the low cirMIS group, and in CD patients, the HR was 1.71 (0.34,8.58, p=0.5] times higher than the low cirMIS group. (G) UNIFI adult UC cohort and the odd's ratio of being in response at week 44 (outcome=clinical remission) in patients considered responders at week 8 according to various definitions from clinical remission (n=33), endoscopic improvement (44), histological healing (65) and HEMH (34) (see methods), based on their baseline high versus low cirMIS status, vs their week 8 Total Mayo score. An additive model was used. +p<0.1; *p<0.05; **p<0.01; ***p<0.001. bMIS, biopsy molecular inflammation score; cirMIS, circulating MIS; HEMH, histoendoscopic mucosal healing; IFX infliximab; MSCCR, Mount Sinai Crohn's and Colitis Registry; TNF, tumour necrosis factor; UST, ustekinumab; VDZ, vedolizumab.



C.

bMIS IBD Transcription Factor Enrichment

Top TF Motif	Motif id	NES	Transcription factor
M1	transfac_public-M00054	6.493	NFKB1,RELA,NFKB2,OVOL2,BCL3,REL,AP3B1,CHURC1,EBF1, STAT6,RELB
M2	transfac_pro-M00924	5.175	JUNB,JUN,FOSL2,FOS,FOSL1,JUND,FOSB,SOX4,ZNF333,BATF, BACH2
M3	transfac_pro-M03580	4.916	SMAD2

Top ChIP-seq tracks (ENCODE)	Track id	NES	Transcription factor
T1	wgEncodeAwgTfbsSydhMcf10aesStat3Etoh01bUniPk	7.309	STAT3
T2	wgEncodeAwgTfbsHaibA549P300V0422111Etoh02UniPk	4.396	EP300
T3	wgEncodeAwgTfbsSydhMcf10aesCfosTam112hHvdUniPk	4.318	FOS

*Minimum NEScore for significance =3
** FDR>0.001
*** Motif/Track rankings 10kb centered around TSS

Figure 7 (A) Summary of the genes according to recurrence in the various molecular inflammation score genesets. (B) Dotpots showing selected top significantly enrichments of the bMIS_IBD geneset in (i) bioplant pathways, (ii) cell type signatures (see methods), (iii) IBD GWAS genes and curated known IBD drug targets. Summary of the magnitude (log 2 Fold enrichment) and significance level (negative log10 BH adjusted p value) are shown. (C) Transcription factor enrichment analysis of the promoters of the bMIS_IBD geneset. The iRegulon application within Cytoscape was used to assess the promoters (10 kb centred around transcription start site) of genes within the bMIS_geneset for enrichment in either transcription factor motifs or ChIP-seq tracks from ENCODE. Normalised enrichment scores (NES) of>3 considered significant with FDR>0.001. The top three scoring motifs or tracks and their associated transcription factors are shown. bMIS, biopsy molecular inflammation score; FDR, false discovery rate.

candidates. (figure 7B, online supplemental table 8). Indeed, there was significant enrichment of bMIS genes in a curated list of anti-IBD gene targets in addition to genes that are reported as IBD GWAS candidates. Curated genesets associated with various intestine-based single cell data sets showed that bMIS-IBD genes are mainly enriched in inflammatory macrophages and monocytes as well as genes coexpressed in module's 4/5 as part of a recently described tissular IBD pathotype defined in patients as non-responsive to therapy and associated with activated fibroblasts with neutrophil-chemoattractant properties. Consistent with this pathotype being associated with potential IL-1 blockade, bMIS-IBD genes were also enriched in genes upregulated in fibroblasts with IL1b treatment. Finally, transcription factor enrichment analysis supports NFKB1, STAT3, JUN/FOS and SMAD2 and their related cofactors as potential transcriptional regulators underlying expression of these disease-associated genes (figure 7C, (online supplemental table 9). Overall, these data support that the molecular score of inflammation may provide a metric to 'quantify' the underlying biological process underlying the patient's disease and thus inform on potential treatment responsiveness and treatment choices.

DISCUSSION

In this study, we derived a new disease activity metric, bMIS, based on expression of genes in the intestinal mucosa from endoscopically inflamed versus non-inflamed biopsies of patients with either CD or UC. We demonstrated that bMIS significantly correlated with current clinical, endoscopic or histologic disease activity assessments. However, this molecular score was (1) more sensitive and granular, detecting disease activity even when the mucosa was histologically normal (2) based on pathobiology which can be customised or generalised by disease type and (3) could be adapted to blood transcriptome data to provide a less invasive biomarker, cirMIS, with similar results as faecal calprotectin and generally better than CRP, the gold-standard disease activity biomarkers. Through seven independent IBD cohorts, bMIS was characterised as being associated with IBD, as well as IBD treatment effects and response, especially cirMIS for CD and bMIS for UC in UST, anti-TNF and VDZ (UC only)-treated cohorts. bMIS was also found to be a stronger prognostic indicator of response to therapy in UC compared with currently used metrics such as the Mayo score. Importantly, we showed that in patients considered macroscopically and microscopically 'normal', but with residual high b/cirMIS (in UC) or high cirMIS (in CD) levels, that rates of relapse were greater, supporting that residual molecular inflammation, beyond any currently used parameters (including histological remission) is clinically important and may portent relapse.

Overcoming the unmet therapeutic needs in patients with IBD will rely not only on novel targets but also on improved robust clinical metrics of disease activity. Our study's goal was to develop a potential molecular diagnostic approach to provide objective evidence and quantification of intestinal inflammation. To this end, we demonstrated strong, significant correlations of bMIS or cirMIS to at least three standard clinical disease activity metrics (clinical, endoscopic and histologic) as well as two biomarkers (faecal calprotectin and CRP) of intestinal inflammation. While we acknowledge that the molecular scores of disease activity are not independent of those used as part of standard care, as they were all originally designed to detect gut inflammation, the reproducible correlations among the molecular scores with different disease activity metrics are important to show, as it demonstrates the potential diagnostic value of bMIS or cirMIS. It is also of interest that the correlations between any existing metric with a molecular score (cirMIS or bMIS) were always higher, than any correlation observed between existing metrics, suggesting the molecular disease assessment, capture a broad disease landscape both cellular, histopathological as well as macroscopic. While it is of note that cirMIS can significantly outperform CRP at distinguishing patients in endoscopic remission, potentially more relevant was the greater effect size observed for cirMIS as compared with CRP levels in distinguishing early treatment responders from non-responders. This effect, observed through blood sampling, was already seen at week 6 samplings, supporting cirMIS as potentially relevant clinical trial outcome, especially in CD, where disease is patchy and biopsy sampling biases exist. To that end, we acknowledge that intestinal molecular scoring faces a potentially similar drawback to histology scoring in patients with CD, in that if a biopsy is not sampled in an area of active disease, the bMIS score of that biopsy will not be able to reflect active inflammation ongoing in the gut elsewhere. In that sense, cirMIS has potentially better utility than bMIS for CD. However, an important point, is that with our molecular scoring platform and less-so for histology, is the possibility of generating akin to cirMIS, a biopsy-level biomarker (geneset) of iMIS, that reflects active intestinal inflammation, regardless of biopsy sampling location. Finally, we observed cirMIS to be as good as faecal calprotectin at distinguishing patients in endoscopic and histologic remission, however, is not reliant on patient stool sampling, which can be challenging to coordinate in the clinic.[47] Importantly, we demonstrate that we could translate the gene sets from the MSCCR discovery cohort and derive equivalently informative bMIS or cirMIS scores in seven independent IBD cohorts. In five of these which were clinical trial cohorts, the molecular score levels were also found to decrease with various treatment (VDZ, UST, or IFX vs placebo) as well as in responders (of various definitions) compared with non-responders.

Optimal management of IBD relies on early intervention, treat-to-target strategies and tight disease control.[9] Using available clinical trial data, we demonstrate that bMIS_UC, in particular, has potential prognostic value, associating significantly better with early response outcomes, than even standard macroscopic assessments (Mayo score). This suggests that molecular scores of inflammation may be able to identify a subset of patients that are actually different, despite being considered similar with respect to macroscopic assessments. Since those patients with the higher bMIS levels appear to have a higher OR of being non-responders, if given the same treatment as the lower bMIS group, this additional insight may guide a physician towards an alternative, potentially more aggressive 'step-down' therapy over typical 'step-up' therapeutic approaches. Thus, molecular scoring systems could provide added value for tighter disease control, better risk stratification and intervention strategies.

Also relevant to treat-to-target strategies is that fact that bMIS and cirMIS were able to sense inflammation through gene expression changes in patients considered to have no (or low) histological or endoscopic abnormalities. This goes to the important follow-up question as to whether molecular disease inactivity plus clinical, endoscopic and histological disease inactivity, 'matters' clinically for optimally managing IBD. Inclusion of endoscopic mucosal healing over resolution of clinical symptoms only has shown to improve prolonged clinical remission.[48 49] This 'deep remission' treatment target was then challenged by 'complete remission' defined by additional microscopic healing. In UC, lack of histological remission has been shown to predict worse clinical outcomes, whereas endoscopic mucosal healing

did not.[50] In patients with ileal CD, histologic ileal healing was also shown to associate more strongly with clinical outcomes, than endoscopic healing.[51] Further trials are ongoing in both UC and CD to continue to evaluate optimal treatment targets.[52–54] Combined these studies suggest undetected inflammation is deleterious implying a need to search for deeper markers of disease control. So how 'deep' should we go? Our substudy in the MSCCR cohort and UNIFI UC clinical trial cohort, showing either higher HR or OR of relapse, respectively, in those patients considered macroscopically and microscopically normal, but with higher molecular inflammation levels, would support that inflammation can be detected molecularly where histologically it is considered low or even normal, and that this matters for long-term clinically relevant outcomes. Admittedly, these latter observations were done in cohorts, of relatively small size (and not available for CD); however, they nonetheless advocate for future studies exploring combinations of molecular and current biomarkers and disease activity indices.

Other published studies support the potential for molecular endpoints, including proteins, as minimally invasive serum biomarkers of IBD outcomes.[45 55] Kessel *et al* described 16-serum proteins that show unstable disease control in patients with IBD with stable remission[45] and a commercial assay of 13-serum protein markers identified patients with resolution of endoscopic disease activity called the endoscopic healing index[55] has also been reported. We noted that several of the 16-serum protein panel were a component of either the bMIS or cirMIS score gene sets. A generally unique feature of molecularly-based disease metrics is that they are protein/gene-centric, meaning they are grounded in the pathophysiological context, thus providing a direct line between clinical indices and biological interpretation. In this sense, our bMIS also informs on the genes/pathways that therapeutics could be aimed against. Key biology associated with the bMIS genesets included signalling by OSM,[46] IL1, IL23 and IL12, Jak-STAT, TGF-beta, IFN-gamma, TNF effects on cytokine activity and apoptosis and G-protein-coupled receptors (GPCR) ligand binding.[56] In addition, cell types seemingly enriched in bMIS genes, and, thus, potentially implicating cell types to target, included inflammatory macrophages, activated fibroblasts and plasma cells, to name a few. Overall, these rich molecular inflammation genesets, strongly linked to clinical outcomes, provides a valuable resource for further mechanistic investigations.

Our study has several strengths and limitations. bMIS/cirMIS could be validated across multiple independent IBD datasets, including those using microarray technology. We were, however, unable to acquire clinical trial data sets to assess clinical outcomes in patients in endoscopic and histologic remission with residual levels of cirMIS for UC or bMIS/cirMIS for CD. We also acknowledge the relatively small numbers of patients when looking at long-term outcomes. Thus, the true clinical significance of residual 'molecular inflammation' remains to be established in appropriately designed longer term cohorts. Furthermore, it will be of interest to evaluate whether the blood-based molecular biomarker, cirMIS, is affected by other factors such as active extraintestinal manifestations of IBD or infection, as is observed with CRP. Also, although the geneset sizes comprising the molecular scores are relatively small, before clinical test implementation, optimisation of the gene sets to maximise detection of molecular inflammation with the fewest number of gene measures would be needed. Also important is determination of the delta or change in the score needed for potential patient outcome improvement (ie, the optimal molecular 'target'). Finally, a strength of our study is that a general IBD molecular score of inflammation was as effective as an IBD-type-specific molecular score, suggesting that the underlying inflammation processes are similar regardless of disease type or location. Indeed, we observed that the correlation of the SESCD subscore for the ileum was 0.56 to bMIS scores within ileal samples. However, our platform could be optimised further to generate molecular scores of disease, which are based on gene sets created from various subclassifications of patients, which could augment potential precision medicine strategies.

In conclusion, we provide a framework for generating a mucosal centric molecular score of inflammation, which provides another layer of disease activity in IBD. Our method provides an opportunity to assess mucosal inflammation in a novel and complementary way, which potentially addresses the issue of persistent microscopic inflammation that escapes current clinical evaluation. Our data suggest that molecular metrics of disease activity could add further stratification to disease activity and may be better predictive markers of response and relapse than currently used clinical measures.

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REFERENCES

1 Kobayashi T, Siegmund B, Le Berre C, *et al*. Ulcerative colitis. *Nat Rev Dis Primers* 2020;6:74.
2 Roda G, Chien Ng S, Kotze PG, *et al*. Crohn's disease. *Nat Rev Dis Primers* 2020;6:22.
3 Ungaro R, Colombel J-F, Lissoos T, *et al*. A Treat-to-Target update in ulcerative colitis: a systematic review. *Am J Gastroenterol* 2019;114:874-883:874–83.
4 Peyrin-Biroulet L, Sandborn W, Sands BE, *et al*. Selecting therapeutic targets in inflammatory bowel disease (STRIDE): determining therapeutic goals for Treat-to-Target. *Am J Gastroenterol* 2015;110:1324–38.
5 Le Berre C, Peyrin-Biroulet L, SPIRIT-IOIBD study group. Selecting end points for disease-modification trials in inflammatory bowel disease: the spirit consensus from the IOIBD. *Gastroenterology* 2021;160:e21:1460.
6 Adegbola SO, Sahnan K, Warusavitarne J, *et al*. Anti-TNF therapy in Crohn's disease. *Int J Mol Sci* 2018;19. doi:10.3390/ijms19082244. [Epub ahead of print: 31 Jul 2018].
7 Li K, Marano C, Zhang H, *et al*. Relationship between combined histologic and endoscopic endpoints and efficacy of ustekinumab treatment in patients with ulcerative colitis. *Gastroenterology* 2020;159:2052–64.
8 Agrawal M, Colombel J-F. Treat-to-Target in inflammatory bowel diseases, what is the target and how do we treat? *Gastrointest Endosc Clin N Am* 2019;29:421–36.
9 Turner D, Ricciuto A, Lewis A, *et al*. STRIDE-II: an update on the selecting therapeutic targets in inflammatory bowel disease (STRIDE) initiative of the International organization for the study of IBD (IOIBD): determining therapeutic goals for Treat-to-Target strategies in IBD. *Gastroenterology* 2021;160:1570–83.
10 Hamilton JD, Suárez-Fariñas M, Dhingra N, *et al*. Dupilumab improves the molecular signature in skin of patients with moderate-to-severe atopic dermatitis. *J Allergy Clin Immunol* 2014;134:1293–300.
11 Guttman-Yassky E, Ungar B, Malik K, *et al*. Molecular signatures order the potency of topically applied anti-inflammatory drugs in patients with atopic dermatitis. *J Allergy Clin Immunol* 2017;140:e13.
12 Beck LA, Thaçi D, Hamilton JD, *et al*. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014;371:130–9.
13 Kosoy R, Kim-Schulze S, Rahman A, *et al*. Deep analysis of the peripheral immune system in IBD reveals new insight in disease subtyping and response to monotherapy or combination therapy. *Cell Mol Gastroenterol Hepatol* 2021;12:599-632.
14 Daperno M, D'Haens G, Van Assche G, *et al*. Development and validation of a new, simplified endoscopic activity score for Crohn's disease: the SES-CD. *Gastrointest Endosc* 2004;60:505–12.
15 Sipponen T, Nuutinen H, Turunen U, *et al*. Endoscopic evaluation of Crohn's disease activity: comparison of the CDEIS and the SES-CD. *Inflamm Bowel Dis* 2010;16:2131–6.
16 Saigusa K, Matsuoka K, Sugimoto S, *et al*. Ulcerative colitis endoscopic index of severity is associated with long-term prognosis in ulcerative colitis patients treated with infliximab. *Dig Endosc* 2016;28:665–70.
17 Marchal-Bressenot A, Salleron J, Boulagnon-Rombi C, *et al*. Development and validation of the Nancy histological index for UC. *Gut* 2017;66:43–9.
18 D'Haens GR, Geboes K, Peeters M, *et al*. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology* 1998;114:262–7.
19 Geboes K, Riddell R, Ost A, *et al*. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. *Gut* 2000;47:404–9.
20 Novak G, Parker CE, Pai RK, *et al*. Histologic scoring indices for evaluation of disease activity in Crohn's disease. *Cochrane Database Syst Rev* 2017;7:CD012351.
21 Li K, Friedman JR, Chan D, *et al*. Effects of Ustekinumab on Histologic Disease Activity in Patients With Crohn's Disease. *Gastroenterology* 2019;157:1019–31.
22 Ma C, Sedano R, Almradi A, *et al*. An international consensus to standardize integration of histopathology in ulcerative colitis clinical trials. *Gastroenterology* 2021;160:2291–302.
23 Satsangi J, Silverberg MS, Vermeire S, *et al*. The Montreal classification of inflammatory bowel disease: controversies, consensus, and implications. *Gut* 2006;55:749–53.
24 Haberman Y, Tickle TL, Dexheimer PJ, *et al*. Pediatric Crohn disease patients exhibit specific ileal transcriptome and microbiome signature. *J Clin Invest* 2014;124:3617–33 https://www.jci.org/articles/view/75436
25 Peters LA, Perrigoue J, Mortha A, *et al*. A functional genomics predictive network model identifies regulators of inflammatory bowel disease. *Nat Genet* 2017;49:1437–49 https://www.nature.com/articles/ng.3947
26 Arijs I, De Hertogh G, Lemmens B, *et al*. Effect of vedolizumab (anti-α4β7-integrin) therapy on histological healing and mucosal gene expression in patients with UC. *Gut* 2018;67:43–52.
27 Sands BE, Sandborn WJ, Panaccione R, *et al*. Ustekinumab as induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2019;381:1201–14.
28 Feagan BG, Sandborn WJ, Gasink C, *et al*. Ustekinumab as induction and maintenance therapy for Crohn's disease. *N Engl J Med* 2016;375:1946–60.
29 Toedter G, Li K, Marano C, *et al*. Gene expression profiling and response signatures associated with differential responses to infliximab treatment in ulcerative colitis. *Am J Gastroenterol* 2011;106:1272–80.
30 Suárez-Fariñas M, Tokuyama M, Wei G, *et al*. Intestinal inflammation modulates the expression of ACE2 and TMPRSS2 and potentially overlaps with the pathogenesis of SARS-CoV-2-related disease. *Gastroenterology* 2021;160:287–301.
31 Di'Narzo AF, Houten SM, Kosoy R, *et al*. Integrative analysis of the inflammatory bowel disease serum metabolome improves our understanding of genetic etiology and points to novel putative therapeutic targets. *Gastroenterology* 2022;162:e11.
32 Hänzelmann S, Castelo R, Guinney J. GSVA: gene set variation analysis for microarray and RNA-seq data. *BMC Bioinformatics* 2013;14:7.
33 Lee E, Chuang H-Y, Kim J-W, *et al*. Inferring pathway activity toward precise disease classification. *PLoS Comput Biol* 2008;4:e1000217.
34 R Development Core Team. R: a language and environment for statistical computing. v3.0.3 EDN (R foundation for statistical computing, 2020).
35 Kuleshov MV, Jones MR, Rouillard AD, *et al*. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res* 2016;44:W90–7.
36 Martin JC, Chang C, Boschetti G, *et al*. Single-Cell analysis of Crohn's disease lesions identifies a pathogenic cellular module associated with resistance to anti-TNF therapy. *Cell* 2019;178:1493–508.
37 Smillie CS, Biton M, Ordovas-Montanes J, *et al*. Intra- and Inter-cellular rewiring of the human colon during ulcerative colitis. *Cell* 2019;178:714–30.

38 Friedrich M, Pohin M, Jackson MA, *et al*. IL-1-driven stromal-neutrophil interactions define a subset of patients with inflammatory bowel disease that does not respond to therapies. *Nat Med* 2021;27:1970–81.
39 Liu JZ, van Sommeren S, Huang H, *et al*. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. *Nat Genet* 2015;47:979–86.
40 Jostins L, Ripke S, Weersma RK, *et al*. Host–microbe interactions have shaped the genetic architecture of inflammatory bowel disease. *Nature* 2012;491:119–24.
41 de Lange KM, Moutsianas L, Lee JC, *et al*. Genome-wide association study implicates immune activation of multiple integrin genes in inflammatory bowel disease. *Nat Genet* 2017;49:256–61.
42 Schreiner P, Neurath MF, Ng SC, *et al*. Mechanism-Based treatment strategies for IBD: cytokines, cell adhesion molecules, JAK inhibitors, gut flora, and more. *Inflamm Intest Dis* 2019;4:79–96.
43 Janky Rekin's, Verfaillie A, Imrichová H, *et al*. iRegulon: from a gene list to a gene regulatory network using large motif and track collections. *PLoS Comput Biol* 2014;10:e1003731.
44 Shannon P, Markiel A, Ozier O, *et al*. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;13:2498–504.
45 Kessel C, Lavric M, Weinhage T, *et al*. Serum biomarkers confirming stable remission in inflammatory bowel disease. *Sci Rep* 2021;11:6690.
46 West NR, Hegazy AN, Owens BMJ, *et al*. Oncostatin M drives intestinal inflammation and predicts response to tumor necrosis factor-neutralizing therapy in patients with inflammatory bowel disease. *Nat Med* 2017;23:579–89.
47 Buisson A, Gonzalez F, Poullenot F, *et al*. Comparative acceptability and perceived clinical utility of monitoring tools: a nationwide survey of patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2017;23:1425–33.
48 Colombel J-F, Rutgeerts PJ, Sandborn WJ, *et al*. Adalimumab induces deep remission in patients with Crohn's disease. *Clin Gastroenterol Hepatol* 2014;12:414–22.
49 Reinisch W, Van Assche G, Befrits R, *et al*. Recommendations for the treatment of ulcerative colitis with infliximab: a gastroenterology expert group consensus. *J Crohns Colitis* 2012;6:248–58.
50 Vuitton L, Peyrin-Biroulet L, Colombel JF, *et al*. Defining endoscopic response and remission in ulcerative colitis clinical trials: an international consensus. *Aliment Pharmacol Ther* 2017;45:801–13.
51 Christensen B, Erlich J, Gibson PR, *et al*. Histologic healing is more strongly associated with clinical outcomes in ileal Crohn's disease than endoscopic healing. *Clin Gastroenterol Hepatol* 2020;18:2518–25.
52 Bryant RV, Burger DC, Delo J, *et al*. Beyond endoscopic mucosal healing in UC: histological remission better predicts corticosteroid use and hospitalisation over 6 years of follow-up. *Gut* 2016;65:408–14.
53 Enhanced algorithm for Crohn's treatment incorporating early combination therapy (REACT2).
54 Determination of the optimal treatment target in ulcerative colitis (verdict) 2021.
55 D'Haens G, Kelly O, Battat R, *et al*. Development and Validation of a Test to Monitor Endoscopic Activity in Patients With Crohn's Disease Based on Serum Levels of Proteins. *Gastroenterology* 2020;158:e10.
56 Olivera P, Danese S, Peyrin-Biroulet L. Next generation of small molecules in inflammatory bowel disease. *Gut* 2017;66:199–209.