




Longitudinal monitoring of STAT3 phosphorylation and histologic outcome of tofacitinib therapy in patients with ulcerative colitis

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Summary

Background: Tofacitinib is the first in class, pan-JAK inhibitor approved for ulcerative colitis (UC). Clinical efficacy has been shown, but long-term real-life endoscopic and histologic data are lacking.

Aim: To investigate the effects of tofacitinib in patients with refractory UC focussing on endoscopic, histologic and molecular outcomes, including STAT3 phosphorylation (pSTAT3) detection in the spatial context of mucosal inflammation

Methods: We prospectively monitored 59 highly refractory patients (96.7% anti-TNF exposure, 91.7% vedolizumab exposure) initiating tofacitinib at two IBD referral centres and assessed outcome at the end of induction and after 48 weeks of therapy. Endoscopic improvement was defined as a Mayo endoscopic subscore ≤ 1 , endoscopic and histologic remission as Mayo endoscopic subscore 0 and Nancy histologic score 0. Multiplex immunohistochemistry with multispectral imaging was used to assess pSTAT3.

Results: Endoscopic improvement was achieved by 24.4% and 30.5% of patients at weeks 8 and 48, respectively. Endoscopic and histologic remission rates were 11.1%, 23.7 and 16.7%, 21.4%, respectively. Endoscopic improvement at week 8 was significantly associated with treatment continuation in the long-term (72.7% vs 20.6%, $p = 0.003$). Although we observed a gradual decrease of mucosal pSTAT3 levels in both remitters and non-remitters ($p < 0.05$), no association with treatment outcome could be demonstrated. However, lamina propria pSTAT3 was significantly associated with the Nancy Histologic index ($p = 0.004$).

Conclusion: Tofacitinib can induce and maintain endoscopic and histologic remission in up to one-quarter of highly refractory UC patients. Longitudinal monitoring of nuclear pSTAT3 in mucosal tissue compartments reflects distinctive on-target effects, independently of long-term treatment outcomes.

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1 | INTRODUCTION

The Janus kinase (JAK) signal transducer and activator of transcription (STAT)–pathway plays a key role in intestinal homeostasis and inflammation.¹ When activated by cytokine signalling, JAKs contribute to the phosphorylation and activation of STAT proteins, which on their turn bind regulatory DNA fragments to promote immune activation and inflammation.² Drugs targeting the JAK/STAT pathway in the treatment of immune-mediated diseases including inflammatory bowel disease (IBD) have been developed over recent years. Ever since, tofacitinib is the first in class pan-JAK inhibitor approved for moderate-to-severe ulcerative colitis (UC).³ Tofacitinib inhibits JAK1, JAK2, JAK3 and TYK2 and modulates STAT1, STAT3, STAT4 and STAT5 phosphorylation.¹

The mechanism of action (MoA) of tofacitinib is mediated via inhibition of JAK1 heterodimers, resulting in blockade of JAK–STAT signalling in various cell types within the inflamed intestinal mucosa. It has been previously shown for other targeted therapies, such as IL-6 trans-signalling inhibition, that efficient target engagement at the site of inflammation is associated with clinical remission.⁴

With evolving treatment targets from clinical remission towards endoscopic and histologic remission,⁵ data reporting efficacy rates of endoscopic and histologic endpoints become increasingly important. Real-life studies on efficacy and safety of tofacitinib in refractory patients are limited,^{6–12} especially in terms of endoscopic outcomes beyond induction. Furthermore, real-life data on histologic outcomes are currently non-existing. In the current study, we focussed on endoscopic and histologic efficacy in refractory UC patients treated with tofacitinib therapy and investigated if molecular markers, are directly linked to the JAK/STAT pathway in the context of tofacitinib exposure, do correlate with outcome and could discriminate responders from non-responders. We performed multiplex immunohistochemistry to comprehensively visualise the cellular microenvironment of the intestinal mucosa, where the density and distribution of stained cells were represented in multiplexed or single-channel display for each cell subtype. To assess the target engagement of tofacitinib, we monitored the phosphorylation of STAT3, a common downstream target of JAK signalling and major mediator of a plethora of pro-inflammatory and pro-regenerative cytokine responses in the intestinal epithelium and surrounding lamina propria cells of the inflamed sigmoid mucosa.^{13–15}

2 | METHODS

2.1 | Study design and patients

This prospective cohort study was conducted at the University Hospitals Leuven (Leuven, Belgium) and the University Hospital Schleswig-Holstein (Kiel, Germany). All included patients had given written consent to participate in the Institutional Review Board

approved IBD Biobank (B322201213950/S53684—Leuven and D489/20 (Kiel)).

All consecutive UC patients who initiated tofacitinib between April 2018 and July 2020 were prospectively included. Patients with ileal pouch-anal anastomosis (IPAA) or without objective active disease (i.e. Mayo endoscopic subscore <2) at baseline were excluded. Patients received tofacitinib 10 mg BID till week 8, with subsequent de-escalation to 5 mg BID. However, patients could receive extended 10 mg BID high dose induction till week 16, upon the discretion of the treating physician. Latent tuberculosis was excluded via chest X-ray and tuberculin skin test or interferon-gamma release assay in all patients, before first tofacitinib administration.

2.2 | Outcomes

Endoscopic remission was defined as a Mayo endoscopic subscore of 0, endoscopic improvement as a Mayo endoscopic subscore of ≤1, and histologic remission as a Nancy histology index of 0. A combination of endoscopic and histologic remission was referred to as mucosal healing. Steroid-free clinical remission was defined as a partial Mayo score of ≤2, with no single subscore >1 and without any steroid exposure, including topical, at assessment. All outcomes were assessed at week 8, week 16 and week 48. Primary non-response was defined as no endoscopic improvement by week 16, whereas loss-of-response (LOR) was defined as endoscopic deterioration (Mayo endoscopic subscore >1) following initial endoscopic improvement by week 16.

2.3 | Multiplexed immunohistochemistry

Three-micrometer sections cut from formalin-fixed, paraffin-embedded (FFPE) tissue blocks were used for multiplexed immunohistochemistry (mIHC) analysis. Biopsy samples were obtained before (baseline) and at 8, 16, 24 or 48 weeks after treatment initiation. At baseline, biopsies were taken at the most inflamed area, and subsequently at the same region. The data set subjected to mIHC included 31 patients with 29 available baseline data points.

OPAL Multiplex Immunohistochemistry Assay kit (Akoya-Biosciences) was used for spectral library generation and data acquisition following the manufacturer's instructions (Development Guide, AkoyaBiosciences). Primary antibodies used for staining included: anti-CD3 (DAKO, clone A0452), anti-CD20 (DAKO, clone M0755), anti-CD15 (Thermo Fisher Scientific, clone 4E10), anti-CD68 (DAKO, clone PGM-1), anti-pSTAT3 (Cell Signalling, clone D3A7) and anti-Pan-Cytokeratin (DAKO, clone AE1/3). Nuclear staining was performed by DAPI (AkoyaBiosciences). Slides were scanned at 20× magnification using “Vectra Polaris” (AkoyaBiosciences) platform. Spectral unmixing and further tissue and cell segmentation, as well as phenotyping, were done using the inForm Advanced Image Analysis software (inForm v2.4.10; AkoyaBiosciences).

2.4 | Statistical analysis

All clinical analyses were performed in R version 4.1.0 (R Development Core Team). Categorical variables were expressed in absolute numbers and relative percentages, whereas continuous variables were expressed as median and interquartile range (IQR). Unpaired data were compared using the Fisher's exact for categorical variables, and the Mann-Whitney U-test for continuous variables. The Wilcoxon signed-rank test was used to compare continuous paired data. A non-response imputation was performed in patients discontinuing therapy prior to the assessment at year 1. Likewise, a last-observation carried forward approach was applied in patients discontinuing tofacitinib prior to year 1. Time to event data were analysed using the Kaplan-Meier method. To identify independent predictors of outcome, stepwise forward addition-backwards elimination binary logistic modelling was applied. Final model selection was based on the most optimal second-order Akaike information criterion.¹⁶ The accepted significance level was $p < 0.05$.

2.5 | Multiplex data analyses

Data output from inForm 2.4.10 (AkoyaBiosciences) was further processed in RStudio (Rstudio v1.1.456) using "R" packages "Phenoptr" and "PhenoptrReports" (Kent S Johnson [2019]) with R version 4.1.0. Consolidated results for "Cell densities" were exported and used for further analyses.

Statistical analyses were performed using GraphPad Prism v8 software (GraphPad Software Inc.). Log-transformation of absolute counts of defined objects was done to normalise the distribution of measurements within different groups. Ordinary one-way ANOVA with Dunnett's multiple comparison test was used to analyse differences between baseline and further time points. To evaluate the statistical correlation between the Nancy Index and multiplexed immunophenotyping, Spearman correlation analysis was applied and all samples were divided into a low disease activity group (Nancy Index "0-2") and high disease activity group (Nancy Index "3-4"). For this analysis, time after therapy initiation was not considered. To account for non-equal variances, the unpaired t-test with Welch's correction was used. The accepted significance level was $p < 0.05$.

3 | RESULTS

3.1 | Study cohort

From April 2018 till July 2020, 59 highly refractory UC patients initiating tofacitinib therapy were recruited at both centres, with a median follow-up of 117.6 (99.4-132.6) weeks. None of the included patients had been exposed to tofacitinib before. All but one (96.7%) had been treated with at least one anti-TNF agent, with 52.5% of them with two anti-TNF agents (Table 1). In addition, 91.7% of patients had also been exposed to vedolizumab before. At tofacitinib

TABLE 1 Baseline characteristics of all included patients

Sex, women, n (%)	23 (39.0)
Age at initiation of tofacitinib, y, median [IQR]	45.4 (34.3-53.2)
Disease duration, y, median [IQR]	7.6 (3.8-17.0)
Faecal calprotectin, µg/g, median [IQR]	1600 (773.0-1800.0)
Serum albumin, g/L, median [IQR]	42.1 (40.3-43.6)
Total Mayo score, median [IQR]	9.0 (8.0-11.0)
Endoscopic scoring	
Mayo endoscopic sub-score, median [IQR]	3.0 (3.0-3.0)
Histologic scoring	
Nancy index, median [IQR]	3.0 (3.0-3.0)
Disease extent, n (%)	
Proctitis E1	8 (13.5)
Left-sided colitis E2	28 (47.5)
Extensive colitis E3	23 (39.0)
Concomitant medication, n (%)	
5-aminosalicylates	27 (45.7)
Corticosteroids	
Topical	17 (28.8)
Systemic	20 (33.9)
Immunomodulators	0 (0.0)
Number of previous biological therapies, median [IQR]	2.0 (2.0-3.0)
Previous therapies, n (%)	
Anti-TNF agents	58 (96.7)
One anti-TNF agent	27 (45.7)
Two anti-TNF agents	31 (52.5)
Vedolizumab	55 (91.7)
Anti-IL12/23p40 or anti-IL23p19	4 (6.8)
Smoking status, n (%)	
Active smoking	14 (23.7)
Previously smoking	5 (8.5)
Never smoked	40 (67.8)
Cardiovascular comorbidity or history of venous thromboembolism, n (%)	10 (16.9)
Statin use, n (%)	6 (10.2)

Abbreviations: n = number of patients, IQR = interquartile range, UCEIS = ulcerative colitis endoscopic index of severity.

initiation, 45.7% of patients received concomitant 5-aminosalicylates, whereas none received immunomodulators (azathioprine, mercaptopurine or methotrexate). At tofacitinib initiation, 33.9% of patients had concomitantly administered systemic corticosteroids, and 28.8% of patients received topical steroid therapy at induction.

3.2 | Clinical, endoscopic and histologic outcomes at the end of induction

Despite a refractory and highly inflammatory phenotype, 20.0% of patients achieved steroid-free clinical remission by week 8. Likewise,

endoscopic improvement and remission were observed in 24.4% and 11.1% of patients, respectively. By week 8, histologic remission and mucosal healing rates were 16.7% and 9.5%, respectively.

Upon discretion of the treating physician, tofacitinib could be tapered (5 mg BID) at week 8 or continued at a higher dose of 10 mg BID till week 16. In the 53 patients who continued therapy beyond week 8, prolonged high dose induction was continued in 34.0% of patients till week 16. This subsequently resulted in a week 16 steroid-free clinical remission rate of 33.3%, as well as endoscopic improvement and remission rates of 31.1% and 15.6%, respectively. In line, a week 16 histologic remission and mucosal healing rates of 26.2% and 11.9% could be observed. Nevertheless, 83.3% of patients who required prolonged high dose with 10 mg BID till week 16 ultimately discontinued therapy due to lack of efficacy.

3.3 | Clinical, endoscopic and histologic outcomes at week 48 and beyond

After 48 weeks of tofacitinib therapy, the partial Mayo score decreased significantly from 7.0 [5.0–8.0] down to 4.0 [1.0–7.0] ($p < 0.001$), resulting in a sustained steroid-free clinical remission rate of 32.2% (Figure 1). Out of all 37 patients on baseline steroids, 18.9% were in clinical remission at week 48, all entirely steroid-free. A 30.5% endoscopic improvement and 23.7% endoscopic remission rate could be objectified. Disease location had an impact on therapeutic efficacy, with patients suffering from extensive colitis achieving endoscopic remission rates (8.7%) less often as compared to patients with left-sided colitis (35.7%, $p = 0.04$) or proctitis (25.0%, $p = 0.27$). As the histological remission rate remained largely stable after 1 year (21.4%), the one-year mucosal healing rate was 17.9%.

Two-thirds of patients (64.4%) discontinued tofacitinib therapy after 16.1 [9.2–28.5] weeks of therapy. Reasons for

treatment discontinuation included primary non-response (26 out of 38), secondary loss-of-response (9 out of 38), side-effects (2 out of 38) and high-grade dysplasia (1 out of 38). Fifteen patients (25.4% of the entire study population) subsequently underwent colectomy after 11.4 (3.1–21.0) weeks. In contrast, the vast majority (85.7%) of patients who achieved endoscopic remission by year 1 did not lose response subsequently and continued tofacitinib therapy with a median tofacitinib exposure of 119.1 [103.4–128.9] weeks.

No venous thrombo-embolisms or cancers were observed during tofacitinib exposure. On top, no new safety signals were observed apart from a disseminated varicella-zoster reactivation, invasive nocardiosis and *Pneumocystis jiroveci* pneumonia in a single patient, as reported earlier.¹⁷

3.4 | Phenotypic features predictive for long-term outcome and treatment continuation

Multivariable logistic regression on baseline phenotypical features (age, disease duration, partial Mayo score, endoscopic Mayo score, serum albumin, faecal calprotectin, Nancy histologic index and total number of biological therapies exposed to) identified age (OR 1.02, $p = 0.01$), baseline serum albumin (OR 1.08, $p = 0.006$) and baseline Mayo endoscopic subscore (OR 0.58, $p = 0.009$) as significantly associated with endoscopic remission after 1 year of tofacitinib therapy (Table 2). On top, age (OR 0.98, $p = 4.6 \times 10^{-5}$) and baseline serum albumin (OR 0.87, $p = 4.8 \times 10^{-6}$) also significantly associated with treatment discontinuation overall. Endoscopic improvement at week 8 was significantly associated with endoscopic remission at week 48 (54.5% vs 17.6%, $p = 0.04$) and treatment continuation on the long-term (72.7% vs 20.6%, $p = 0.003$).

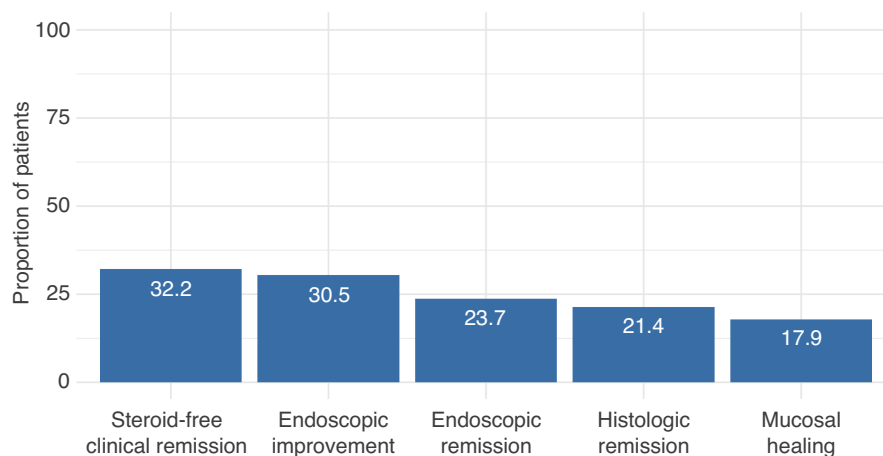


FIGURE 1 Outcomes after 48 weeks of tofacitinib therapy in refractory UC patients. Steroid-free clinical remission was defined as a partial Mayo score of ≤ 2 , with no single subscore > 1 and without any steroid exposure at assessment. Endoscopic improvement was defined as a Mayo endoscopic subscore of ≤ 1 , endoscopic remission as a Mayo endoscopic subscore of 0. Histologic remission was defined as a Nancy histology index of 0. A combination of endoscopic and histologic remission was referred to as mucosal healing. $N = 59$ for clinical and endoscopic outcomes, $n = 56$ for histologic outcomes

TABLE 2 Overview of the baseline predictors of the best model of endoscopic remission at year 1

	Odds ratio
Age, years*	1.02 (1.00–1.03)
Serum albumin (g/dL)**	1.08 (1.02–1.14)
Mayo endoscopic subscore**	0.58 (0.40–0.82)
Nancy histologic index	1.05 (0.89–1.24)
Disease duration (years)	–
Partial Mayo score	–
Faecal calprotectin (µg/g)	–
Total number of biologicals before	–

Note: Data represent estimated odds ratio with a 95% confidence interval. Variance inflation factors are all below 2. Statistically significant predictors are in bold. * $p < 0.05$, ** $p < 0.01$. Final model selection was based on the most optimal second-order Akaike information criterion (AIC_c).

3.5 | Multiplexed immunohistochemistry identifies decrease of pSTAT3 levels in response to tofacitinib treatment across tissue compartments

The putative mechanism of action of tofacitinib is mediated via efficient blockade of JAK–STAT signalling in various cell types within the inflamed intestinal mucosa. Baseline samples predominantly showed dense cell areas enriched with pSTAT3 positive and negative immune cells (consisting of dispersed lymphocytes and granulocytes), which simultaneously infiltrated the epithelial lining (Figure 2).

To assess whether tofacitinib treatment is associated with inhibition of mucosal pSTAT3 level, we analysed overall, compartment (epithelial vs non-epithelial) and cell-specific pSTAT3 levels at baseline and several time points after therapy induction. Treatment with

tofacitinib demonstrated a global reduction of the density of cells displaying nuclear pSTAT3 staining at week 16 among almost all analysed cell subsets (Figure 3A–I). The monitoring of pSTAT3 density among all available biopsy samples showed an overall reduction already at week 16 ($p = 0.03$, per total tissue, Figure 3B), as well as specifically in the lamina propria ($p = 0.03$, Figure 3C) or in the intestinal epithelium ($p = 0.04$, Figure 3D) compared to baseline levels. Analysis of pSTAT3 levels in lymphocytes (Figure 3E,F) revealed their contribution to the global decrease of density of pSTAT3 positive cells at weeks 16 and 48 after treatment initiation (pSTAT3⁺/CD3⁺: $p = 0.03$ and 0.04 , respectively; pSTAT3⁺/CD20⁺: $p = 0.02$ and 0.03 , respectively). A similar trend was demonstrated by pSTAT3⁺/CD15⁺ cells at week 16 and 24 ($p = 0.05$, and $p = 0.09$, Figure 3G). Whereas analysis of pSTAT3 in CD68⁺ cells did not show significant changes over time (Figure 3H), a pSTAT3 decrease was observed also for cells of undefined cellular identity (pSTAT3⁺/Other) ($p = 0.03$, Figure 3I).

3.6 | Density of mucosal pSTAT3 positive cells reflects severity of mucosal inflammation

We next aimed to identify whether the degree of pSTAT3 levels is a molecular surrogate marker of mucosal inflammation, and therefore correlated relative pSTAT3 levels with the histological Nancy-Index (Table 3). In addition, we assessed the difference in pSTAT3 density using the differentiation of mild vs. moderate to severe acute inflammatory cell infiltrate (samples with Nancy index 0–2 vs 3–4). Both approaches showed the association between pSTAT3 levels overall (Figure S1a) and in various cell types with Nancy-Index. We found that the majority of pSTAT3 responsible for this association was localised in lamina propria ($p = 0.004$, Figure S1b). The most representative cell subsets were pSTAT3⁺/CD15⁺ and pSTAT3⁺/

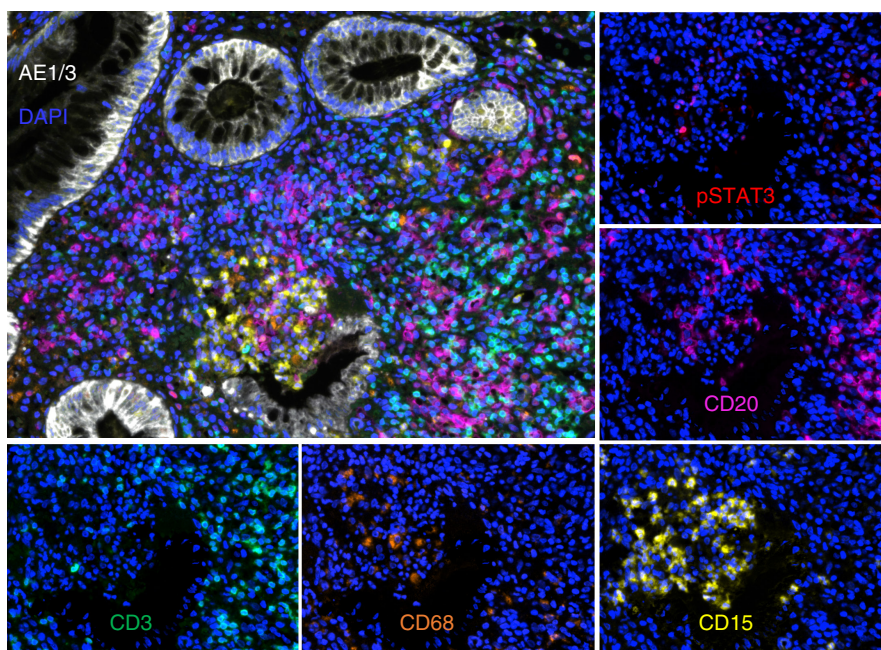
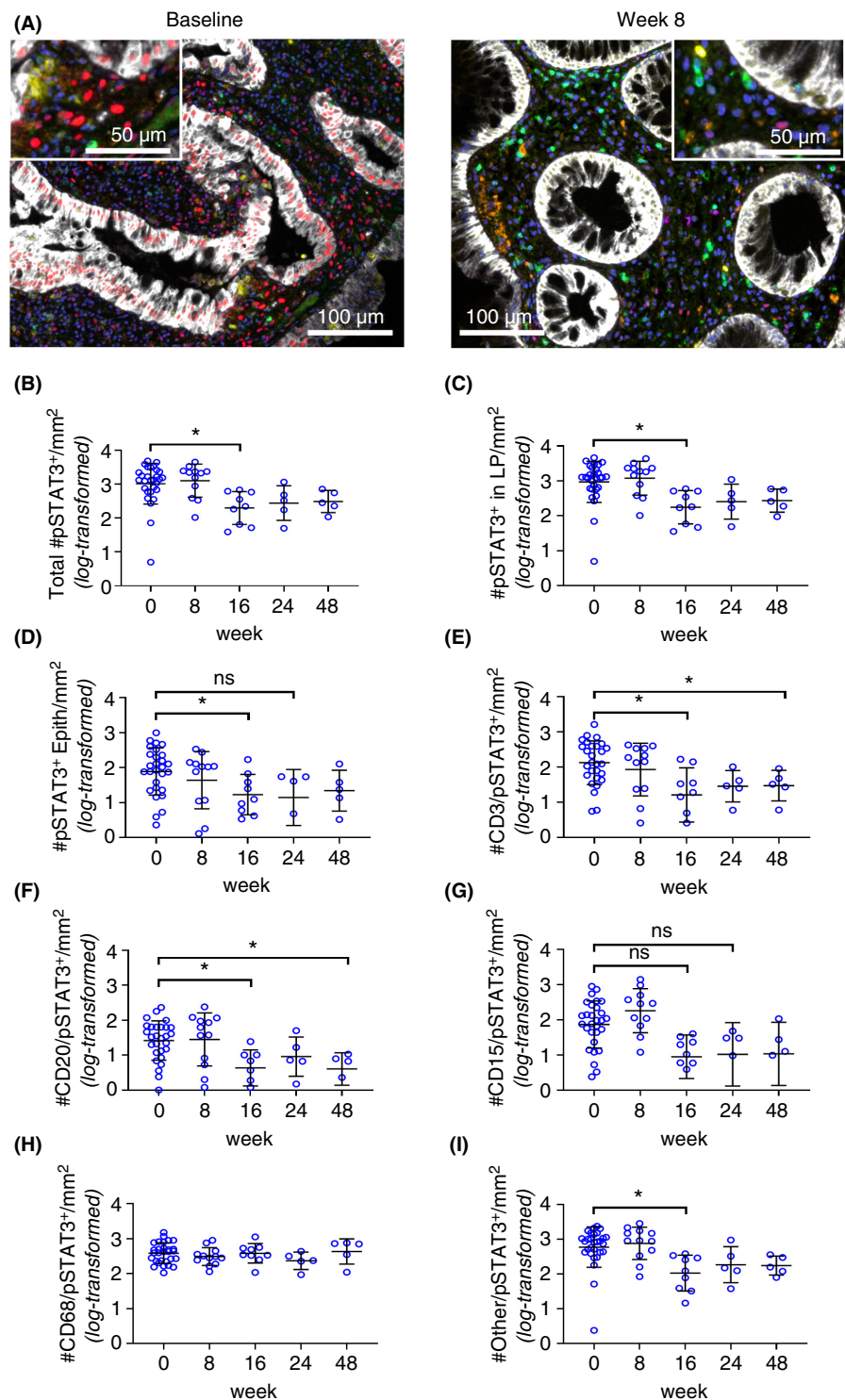


FIGURE 2 Multiplexed immunophenotyping of intestinal biopsies represents the microenvironment of epithelial cells (labelled with pan-cytokeratin AE1/3, white) colocalized with major immune cell populations: Macrophages (CD68⁺, orange), T-lymphocytes (CD3⁺, green), B-lymphocytes (CD20⁺, magenta) and granulocytes (CD15⁺, yellow). Some of cells co-express pSTAT3 (red). Nuclear staining was visualised using DAPI (blue). Whole slide images were obtained at 20× magnification

FIGURE 3 Longitudinal monitoring of pSTAT3 across multiple cell subsets. (A) Representative image of remission case demonstrating a substantial decrease of pSTAT3 (red) at week 8 (right panel) compared to the baseline (left panel) after therapy initiation. Images were taken at 40x magnification, white bar equals 100 μ m (50 μ m for inserts). (B) Total density of pSTAT3⁺ cells per biopsy. (C) Density of pSTAT3⁺ cells in lamina propria (LP), (D) in epithelium and (e) co-expressing CD3, (F) co-expressing CD20, (G) co-expressing CD15, (H) co-expressing CD68 or (I) expressing no markers (CD3[−]/CD20[−]/CD15[−]/CD68[−] non-epithelial cells, pSTAT3⁺/other). Values were measured at baseline, 8, 16, 24 and 48 weeks of treatment ($N = 29$, 12, 9, 5 and 5, respectively). The mixed-effects model with Dunnett's correction for multiple comparison tests were used to evaluate differences between baseline and further time points. Values were log-transformed prior to the analysis. Statistical significance is indicated as ^{ns} $p < 0.1$, * $p < 0.05$



Other, which consists of a heterogeneous cell population with approx. 20–45% of CD34⁺ endothelial cells (Table 3; Figure S1c,d, $p < 0.001$, and $p = 0.01$, respectively). pSTAT3⁺ lymphocytes demonstrated an intermediate association ($p = 0.04$ in CD3, and $p = 0.04$ in CD20, Figure S1e,f), while nuclear pSTAT3⁺ staining in CD68 showed no correlation with Nancy-Index (Table 3, Figure S1g). The presence of pSTAT3⁺ epithelial cells did not show any significant correlation with Nancy-Index (Table 3, Figure S1h).

3.7 | Mucosal pSTAT3 levels are not predictive for therapy response or long-term therapy adherence in tofacitinib treated ulcerative colitis patients

Finally, we aimed to evaluate whether the degree of pSTAT3 staining is a molecular biomarker of short- and long-term responses. Spatially resolved baseline densities of pSTAT3⁺ nuclei were analysed in mucosal epithelium and in lamina propria separately. Obtained data

TABLE 3 Correlation between Nancy index and multiplexed immunohistochemistry analysis output (pSTAT3⁺ cell subsets)

Spearman correlation assay	Nancy index	Nancy index	Nancy index	Nancy index	Nancy index	Nancy index	Nancy index	Nancy index	Nancy index	Nancy index	Nancy index
	vs	vs	vs	vs	vs	vs	vs	vs	vs	vs	vs
	CD15	CD15 ⁺ /pSTAT3 ⁺	Total pSTAT3	Epithelium/pSTAT3 ⁺	pSTAT3 in lamina. Pr.	CD3 ⁺ /pSTAT3 ⁺	CD20 ⁺ /pSTAT3 ⁺	CD68 ⁺ /pSTAT3 ⁺	Other/pSTAT3 ⁺		
Correlation coefficient R	0.60	0.33	0.20	0.02	0.22	0.08	0.11	-0.01	0.22		
95% confidence interval	0.41–0.73	0.09–0.52	-0.04 to 0.42	-0.22 to 0.26	-0.03 to 0.44	-0.17 to 0.32	-0.14 to 0.34	-0.25 to 0.24	-0.02 to 0.44		
p value	<0.0001	0.006	0.10	0.87	0.07	0.51	0.38	0.95	0.06		

were organised into groups based on the remission status at week 16 (short-term) and week 48 (long-term). Interestingly, significantly less baseline pSTAT3 was observed within the lamina propria among patients, who reached remission at week 16 ($p = 0.049$, Figure 4b). In contrast, intestinal epithelial pSTAT3 levels did not show a response-specific difference at baseline ($p = 0.72$, Figure 4c). However, stratifying patients using week 48 remission outcome did neither reveal any significant differences in baseline pSTAT3 nuclei staining in the epithelium, nor in lamina propria (data not shown). In addition, we aimed to identify whether longitudinal changes in mucosal STAT3 levels differ between remission/non-remission patients (defined by clinical remission at week 16) or can be used as predictors of long-term therapy response (defined by clinical remission at week 48). We were unable to observe longitudinal changes of mucosal pSTAT3 levels between remission/non-remission changes (Figure S2), nor did we find significant changes in long-term remission status (data not shown).

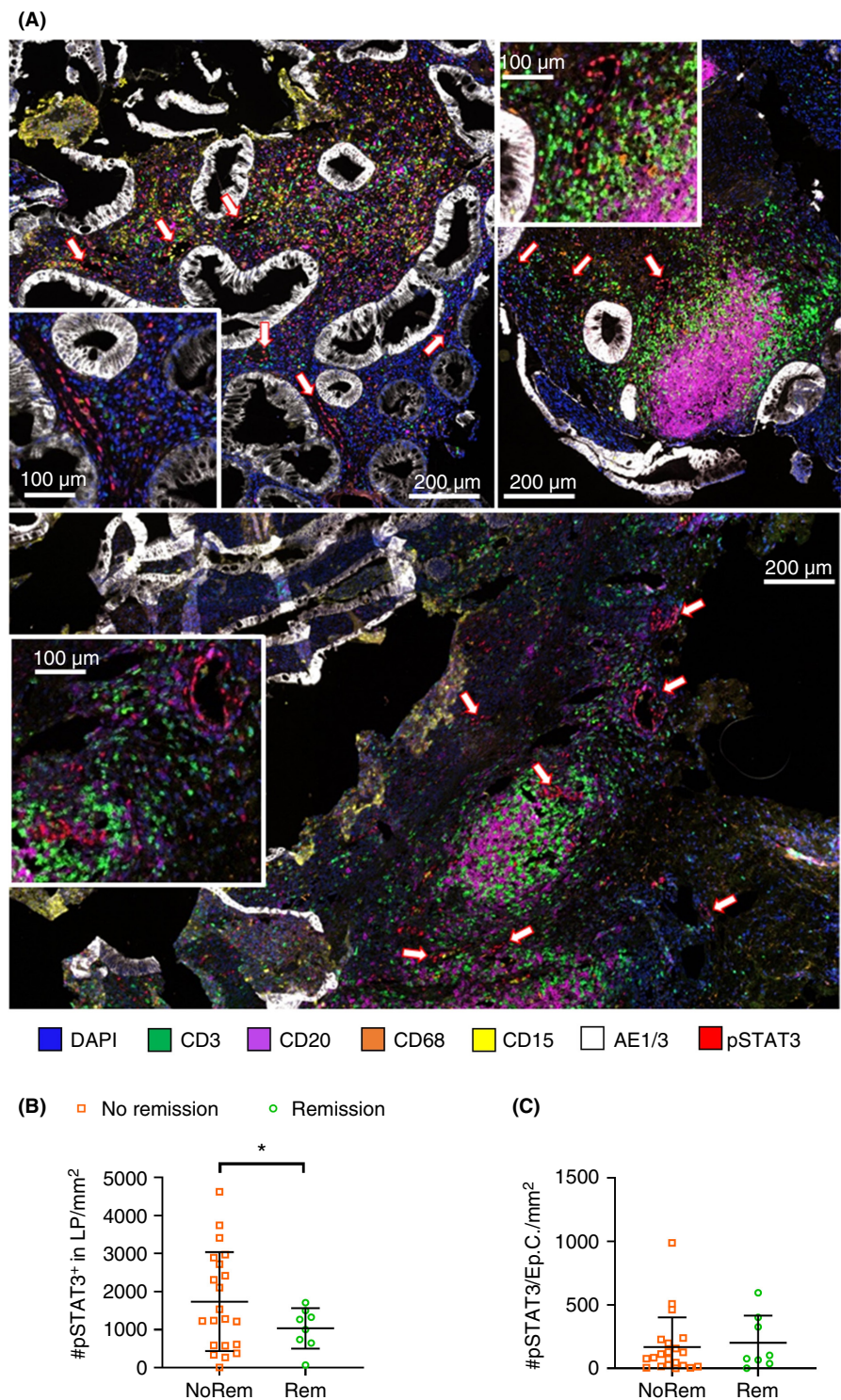
4 | DISCUSSION

Even in highly refractory UC patients, tofacitinib can induce and maintain endoscopic and histologic remission in up to one-quarter of patients. Although not breaking the therapeutic ceiling,¹⁸ it could offer a therapeutic solution and prevent colectomy in a subset of UC patients. Interestingly, the initial effect observed during the first 8 weeks of therapy seems discriminative and a clear indication of sustained response in the long term. 72.7% of patients experiencing endoscopic improvement by week 8 continued therapy on the long-term. Patients who received extended high dose induction ultimately discontinued therapy due to lack of efficacy in the vast majority. This is in contrast to the data from OCTAVE that showed that extended induction resulted in endoscopic remission in 44.6% of patients after 1 year of therapy.¹⁹

Primary non-response rates were high (44.1%) in this refractory cohort, but in line with previous retrospective series,^{7,9,10,12} as was a steroid-free clinical remission rate of 32.2% after 1 year of tofacitinib therapy.^{6,10} In the tofacitinib phase III OCTAVE SUSTAIN development programme, endoscopic improvement rates (Mayo endoscopic subscore ≤ 1 , originally defined as mucosal healing in the original OCTAVE protocols) of 37.4% by week 52 have been observed in patients treated with tofacitinib 5 mg BID.³ Similar to other biological compounds, endoscopic improvement rates at week 52 were higher in anti-TNF naïve (42.6%) versus anti-TNF exposed patients (30.1%).²⁰ Hence, efficacy rates in highly refractory UC patients are expected to be lower than those reported in the landmark trials.

Currently, no validated predictive clinical models exist to estimate the likelihood of future tofacitinib response. In the current cohort study, we identified age, baseline serum albumin and baseline Mayo endoscopic subscore as associative clinical features. However, many of these clinical features have previously been linked with the likelihood of response with other therapeutic compounds,^{21,22} and

FIGURE 4 (A) Images representing pSTAT3 distribution (red dots) pattern at various time points and among different patients. Arrows indicate co-localization of pSTAT3⁺ staining with blood vessels area. Images were taken at 20× magnification, white bar equals 200 μm (100 μm for inserts). Baseline densities of pSTAT3 among patients who got clinical remission (green circles, *N* = 8) vs no remission patients (orange squares, *N* = 21) based on wk16 assessment after therapy initiation in LP (B), in epithelium (C). Unpaired t test with Welch's correction was used to evaluate differences between groups. Statistical significance is indicated **p* < 0.05



therefore likely reflect disease burden and disease severity rather than truly tofacitinib specific predictors.

Subsequently, we assessed the reduction of mucosal pSTAT3 levels during tofacitinib therapy. Our data implicate that the assessment of mucosal pSTAT3 levels might serve as a molecular marker for the assessment of mucosal disease severity, as we observed a strong association of lamina propria pSTAT3 levels with histological disease severity (Nancy-Index). In addition, we found

that mucosal pSTAT3 levels significantly decrease in response to tofacitinib exposure, which might point towards an organ-specific target engagement signature of tofacitinib treatment. However, although a gradual decrease in STAT3 phosphorylation was seen in almost all cell types, it could not be demonstrated as a valid and robust biomarker for tofacitinib long-term efficacy.

Therefore, pSTAT3 assessed by immunohistochemistry is an important, but not completely comprehensive, biomarker.

Nevertheless, our data implicate that tofacitinib leads to a significant reduction of downstream signals of the JAK/STAT pathway in epithelial and non-epithelial cellular compartments of the inflamed colonic mucosa, but as such this reduction is not correlated with clinically important outcomes. Hence, a more detailed, and individualised molecular characterisation will be needed to predict and monitor tofacitinib therapy.²³

Although we acknowledge the relatively small sample size and the lack of central reading for endoscopic and histologic images, we do believe the current study adds to the real-life data on tofacitinib therapy in refractory UC patients. Until now, real-life long-term endoscopic and histologic data were non-existing, as well as the assessment of STAT3 phosphorylation, which becomes increasingly important as a molecular readout in the current treatment-to-target era. In this context, our data indicate that drug-specific assessment of target engagement signatures might be helpful in guiding individualised treatment approaches in UC. On the other hand, these data also underscore the necessity for more fine-grained molecular analysis beyond the JAK-STAT pathway, as the assessment of JAK-STAT signalling events in response to JAK inhibition is not able to disentangle the primary target engagement effect from secondary molecular effects related to the amelioration of proinflammatory events in general. Furthermore, the role of pSTAT3 cannot be generalised and seems to be contrary in different cell types (e.g. lack of STAT3 in intra-epithelial cells promotes colitis).

In conclusion, the current prospective dual-centre cohort study demonstrated that tofacitinib can induce and maintain reproducible endoscopic and histologic response patterns in up to one-quarter of previously refractory UC patients. Although tofacitinib clearly affects the density of mucosal pSTAT3-positive cells in distinct subpopulations, we could not find any association with treatment outcome using immunohistochemistry.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section.

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