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How do we predict a patient's disease course and whether they will respond to specific treatments?

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# Title: How do we predict a patient's disease course and whether they will respond to specific treatments?

Running title: Predicting prognosis and treatment response in IBD

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## Author Contributions

JCL conceived the review. BV, MP and JCL co-wrote and edited the review.

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**Abstract**

Gastroenterologists will be all too familiar with the difficult decisions that managing IBD often presents. How aggressively should I treat this patient? Do I expect them to have a mild or aggressive form of disease? Do they need a biologic? If so, which one? And when should I start it? The reality is that the answers that would be right for one patient might be disastrous for another. The growing therapeutic armamentarium will only make these decisions more difficult, and yet we have seen how other specialties have begun to use the molecular heterogeneity in their diseases to provide some answers. In this article, we review the progress that has been made in predicting the future for any given IBD patient – whether that is the course of disease that they will experience or whether or not they will respond to, or indeed tolerate, a particular therapy.

**Keywords**: inflammatory bowel diseases**,** biomarkers, prognosis, prediction, precision.

## Introduction

Inflammatory bowel disease (IBD) is characterised by a relapsing-remitting course,[1] with heterogenous clinical phenotypes along a continuous disease spectrum (reviewed by Silverberg and colleagues in this issue). The reasons underlying this heterogeneity in clinical presentation and subsequent disease course remain largely unresolved. Indeed, a major challenge in treating IBD has been that at diagnosis, the future disease course is unknown and largely unpredictable.[2, 3] Moreover, even if prognosis could be reliably predicted, it is not currently possible to determine which treatment is most likely to be effective, and least likely to cause toxicity, in an individual patient.[4]

The overarching aim of precision medicine is to ensure that the right treatment is given to the right patient at the right time (**Figure 1**).[5-7] This mantra has, in turn, informed research efforts in IBD, which broadly fall into two main areas. First, there has been considerable focus on identifying prognostic biomarkers. These would help physicians to make informed decisions for individual patients: is a step-up approach most appropriate or is more aggressive treatment required? Where does the balance sit between potent immunosuppression versus the consequences of uncontrolled inflammation? Second, there have been related efforts to identify predictive biomarkers that can indicate – for patients who require biologic therapy, for example – which agent would be most appropriate, balancing the individual's probability of responding with their personal risk of side-effects. This concept of a personalised approach to IBD management has been discussed for years, but has not yet materialised in clinical practice. Indeed, despite recent advances in understanding the molecular basis of immune-mediated diseases, including IBD, treatment still commonly follows a "one-size-fits-all" approach and many decisions are based on clinical tools that have poor predictive performance. Precision medicine might therefore be perceived to be an unrealistic and unachievable goal in IBD, but it is important to note that such an approach is already in clinical use in other fields, notably oncology.[8, 9] While the genetic, phenotypic and molecular variability of IBD may be more complex than cancer, this provides reason for hope, and as Nelson Mandela famously said, "it always seems impossible until it's done!"

So, what do we need to develop to deliver precision medicine in IBD (**Box 1**)? Most importantly, we will need reliable and validated biomarkers to predict three specific

biological features for individual patients: (1) their disease prognosis, (2) their probability of responding to each of the available therapies and (3) their risk of specific drug side-effects or toxicities. In addition, it is clear that no biomarker will be able to predict the future with 100% certainty, so both understanding and being able to communicate risk – a concept that is generally poorly understood – will be important. For example, if a validated biomarker indicates that a patient is 5 times more likely to respond to a treatment, this doesn't mean it is wrong if the patient doesn't respond, just that they were in the minority of patients with that biomarker result who do not respond (**Figure 2**). Effectively communicating the implications of biomarker results to physicians and patients, while also ensuring that emerging biomarkers are robustly developed and validated, will be important steps towards bringing such tools into clinical practice (**Box 1**).

In this article, we review the progress made towards precision medicine in IBD: prediction of disease course and prediction of treatment outcomes (both efficacy and side-effects). We highlight both the successes and challenges in these areas and outline what will be needed to make personalised medicine a reality for our patients.

## Predicting disease course

Being able to reliably predict disease course is a key step in developing a precision-medicine approach for any condition where there is variability in prognosis,[10] including IBD.[11, 12] For example, there is little doubt that conventional "step-up" treatment escalation in response to disease flares is appropriate for patients with an indolent disease course, but would undertreat those destined to have more aggressive disease. Conversely, treating all patients with a "top-down" approach[13] – with powerful immunosuppressants being initiated early in the disease course – would be unaffordable in many healthcare systems and expose patients destined for mild disease to the risks and side-effects of unnecessary treatment. In between, an "accelerated step-up" approach has been suggested, with early step-up to biological agents and small molecules if remission is not promptly achieved with conventional therapy. However, the considerable variability in disease course means that no single treatment strategy is likely to be optimal for all IBD patients. Moreover, aside from considering the potency of the treatment strategy, there is also a need to avoid therapies to which an individual patient may derive minimal benefit or even develop

toxicity. This will also vary between patients, and illustrates why biomarkers to predict treatment response will need to accompany prognostic biomarkers to effectively deliver personalised therapy (**Figure 1**).

Over the years, various prognostic markers have been proposed, but few if any have become embedded in routine clinical care. The reasons for this are multifactorial, but principally relate to lack of clinical utility, itself a consequence of a failure of prospective validation, or confusing statistical associations with prediction **(Box 1)**. We have summarised promising biomarkers that have been validated or are undergoing validation in **Box 2** and highlighted ongoing large-scale biomarker studies that aim to develop or test biomarkers in **Box 3**.

*Clinical prognostic factors* – In both Crohn's disease (CD) and ulcerative colitis (UC), various clinical prognostic factors have been identified from retrospective observational studies. Perianal disease, upper gastrointestinal involvement, requirement for steroids at diagnosis, ileocolonic disease, smoking and severity of index endoscopic disease activity have all been linked with an adverse disease outcome in CD,[14-16] whereas extensive colitis, age at onset or concomitant extra-intestinal manifestations (including PSC) have been linked with worse outcome in UC.[17-19] Some of these associations have been reported in multiple studies, but their predictive performance is poor – a fact that was noted in some of the original reports.[20] This has not deterred clinicians from using these phenotypic features when they treat patients, perhaps because few other tools are available. However, it is clear that clinical characteristics alone cannot reliably guide clinical decision making.

*Genetic prognostic factors* – Following the successful identification of genetic variants that contribute to the development of IBD, there has been a growing interest in whether these same variants might also affect disease course. For example, several studies have examined whether polymorphisms in *NOD2* – the largest genetic risk factor for CD – might influence disease outcome. The results initially appeared promising as a consistent retrospective association was observed between carriage of *NOD2* variants and the need for surgery.[21, 22] *NOD2* variants were subsequently included in composite risk models (in conjunction with clinical and serologic variables) for both paediatric CD,[23] and adult CD to form a web-based, personalised risk and outcome prediction tool (PROSPECT).[24] However, it has since

been shown that *NOD2* variants are specifically associated with ileal CD and that the apparent link with increased need for surgery was in fact driven by the confounding association with disease location. After correcting for this confounding effect, no association between *NOD2* and disease course is observed.[25] Indeed, no susceptibility variant has shown a consistent association with disease course.

One possible explanation for these results is that there is no genetic contribution to prognosis. This, however, would be difficult to reconcile with family studies that have consistently shown similar patterns of disease in affected relatives.[26] Another explanation might be that the genetic variants that contribute to prognosis are different to those that contribute to disease susceptibility, and so wouldn't be found without a broader search. To address this, a genome-wide association study (GWAS) of prognosis in CD was performed in over 2700 patients [27] – comparing patients with frequently flaring, treatment-refractory disease with those with mild disease. Genetic variants at four distinct loci were associated with prognosis (*FOXO3, XACT, IGFBP1*, and the MHC region stretching from *HLA-B* to *HLA-DR* genes). Strikingly, none of these variants had been linked to CD susceptibility, while the sum of all susceptibility variants showed no meaningful association with prognosis. This result, which was confirmed in a subsequent analysis,[28] demonstrated that the principal genetic (and presumably biological) contribution to prognosis is different to disease development. An equivalent study has not yet been performed in UC, but the CD prognosis GWAS already provides clues into the underlying biology of prognosis. For example, the *FOXO3* genetic variant, which had previously been identified in a candidate gene study, has been shown to regulate pro-inflammatory cytokine production in monocytes,[29] while the HLA haplotype has been shown to affect the magnitude of T cell responses in various situations.[30, 31] Despite their functional validation, the low odds ratios of these loci preclude their use as prognostic tests.

This, however, does not mean that genetic tests will not be useful in the future. For example, there is a growing interest in using polygenic risk scores (PRS), which combine weighted allele counts across a large number of variants (even those that fall below the conventional statistical threshold for GWAS significance). Such PRS capture much more genetic risk than individual variants, and have been shown to be useful in other fields. For example, in breast cancer, highly penetrant *BRCA1* and

*BRCA2* mutations account for <25% of the genetic risk of disease, and PRS for common variants have been shown to be able to provide personalised risk assessments to women at high-risk of breast cancer within population screening programs.[32] In IBD, the key question is not whether PRS can predict disease development, but rather whether they could help predict disease prognosis. This will require large scale genetic studies, but may be enabled serendipitously - for example with the increasing use of GWAS arrays in routine clinical care (e.g. for pharmacogenetics or cardiovascular risk stratification).

*Transcriptomics* – In recent years several adult and paediatric studies have identified transcriptional signatures linked to disease course.[33-38] Much focus has been on intestinal transcriptomics, with the RISK (Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with Crohn's Disease) study being a landmark exemplar.[8] In this study, an extracellular matrix tissue transcriptomic signature was identified in 913 paediatric CD patients and shown to predict stricturing disease within the following three years.[34] This was combined with data relating to disease location, antimicrobial serologies, and race to provide a promising risk prediction model for paediatric CD. Importantly, however, this is yet to be independently validated. Furthermore, its predictive performance was assessed using 'leave-one-out' cross-validation – a method that re-samples from the discovery cohort. This carries a risk of overfitting,[39] which is a major reason why many biomarkers fail validation in independent cohorts (**Box 1**).[40] Independent replication is needed before this biomarker can be used clinically. The RISK study has, however, underscored the value of recruiting large, well-characterised, prospective cohorts prior to initiation of treatment; an approach that is increasingly being adopted by multiple (inter)national consortia and biobanking projects (**Box 3**).[41, 42] These should allow for larger discovery and validation datasets that will help advance this field in the near future.

In recent years, focus has turned towards non-invasive blood-based prognostic biomarkers - easier to perform and more acceptable to patients. Data from the IBD Character consortium suggested a potential prognostic role for circulating CD4+ T cell miRNAs,[43] though prospective validation and functional confirmation are awaited. Currently, the only validated prognostic blood test in IBD (PredictSure IBD,

PredictImmune), was derived from a gene-expression signature detected in CD8+ T cells from treatment-naïve patients newly-diagnosed with a variety of immune-mediated disorders, including IBD. This biomarker reliably predicted a more aggressive disease course characterised by early and repeated requirements for treatment escalation.[33, 44, 45] The discovery of this CD8+ T cell gene signature also provided biological insights, highlighting CD8+ T cell exhaustion as a process that leads to fewer disease flares. To develop a practical clinical tool, a lengthy translation process was undertaken to identify genes whose differential expression in whole blood identified analogous patient subgroups to those identified by the CD8+ T cell signature. This resulted in a 17 gene whole blood qPCR classifier, which was subsequently validated in independent cohorts of newly diagnosed CD and UC patients from 4 centres – thereby confirming that it reliably identified patients at high or low risk of future aggressive disease.[37] This blood-based prognostic biomarker is currently being assessed in the PROFILE trial (ISRCTN 11808228), where newly-diagnosed CD patients are stratified based on their biomarker status to assess the most effective treatment strategy (**Box 3**).[46] Importantly, this biomarker has since been shown to be affected by concomitant steroid use, with loss of predictive performance,[47] and so cannot be used if patients are on steroids. A recent paper also suggested that the CD8+ T cell signature was not present in paediatric patients or in a cross-sectional adult cohort.[48] However, both of these findings were subsequently shown to due to methodological issues – with the signature actually being detectable in both.[49]

*Proteomics* – C-reactive protein (CRP) is a well-known marker of systemic inflammation, commonly used to assess disease activity in IBD. In a Scandinavian population-based study, elevated CRP levels were associated with increased need for future surgery in both CD and UC.[50] Similarly, in asymptomatic CD patients, an elevated CRP has been reported to associate with a worse long-term outcome, including more hospitalisations and intestinal resections.[51] However, not all patients with active CD or UC will have an elevated CRP, which is why faecal markers of inflammation – that are more sensitive and specific – are increasingly used in disease assessment.[52] Indeed, serial measurement of faecal calprotectin can also predict relapse.[53, 54] An important caveat with these approaches is that both CRP and faecal calprotectin reflect ongoing inflammation, which unsurprisingly has a greater

chance of progressing to a symptomatic flare-up or disease-related complications than if the patient were truly in remission. Neither are therefore truly predictive, but may be useful if measured serially.

Due to advances in proteomic technologies, researchers are now able to perform broader screens to identify other proteins that might be useful predictively. One recent study from the IBD Character consortium investigated a series of serum proteins that had already been linked to IBD and identified 15 (out of 460 proteins measured) that were associated with the future need for biologics or surgery in an inception cohort of 279 IBD patients. Interestingly, the strongest associations were all observed in UC, with none of these proteins being significantly associated with need for treatment escalation in CD. These results have not yet been independently validated, but other larger proteomic studies are underway, such as PREDICTS (PRoteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects) in North America,[55] and so we should soon know whether these, or other proteomic markers, have clinical utility.

*Microbial* – Given the central role that the microbiome plays in the pathogenesis of IBD, it is unsurprising that studies have investigated whether this might also be useful for predicting disease course – either by measuring the host response to microbial components or the composition of microbiome directly. Several studies have, for example, shown that serological responses to bacterial antigens are more commonly seen in CD patients who have experienced more disease complications.[56, 57] However, the retrospective nature of these studies and observation that seropositivity increases with disease duration raises questions as to whether these are a cause or effect of more aggressive disease. This uncertainty, combined with the limited predictive value of these markers in prospective studies, means that they are not currently recommended for guiding treatment decisions. Other studies have attempted to correlate the abundance of specific species of intestinal bacteria with prognosis in IBD, especially CD. Interpreting such studies is difficult since changes in microbial abundance have been reported with a large number of potential confounders, including inflammation, dietary changes and smoking.[58] Nonetheless, it has been reported that a reduced abundance of a particular *Clostridium* species, *Faecalibacterium prausnitzii*, is associated with a higher rate of post-operative recurrence in CD patients.[59] Resolving the cause-effect

relationship in such situations is challenging, although it is noteworthy that microbiota from IBD patients have been found to exacerbate colitis in mouse models,[60] suggesting that the dysbiosis may not only be a consequence of inflammation. Disentangling the relative contributions of diet, environmental factors and gut microbiota to the course of IBD is a sizeable challenge, but efforts are underway to address this. For example, the PREdiCCt study is prospectively following 1500 IBD patients with detailed dietary and environmental data and allied microbial analysis, aiming to better understand the contributions of these factors (**Box 3**).

## Predicting treatment response and safety

Recent years have seen the late phase development and approval of multiple new therapeutic compounds for the treatment of IBD. Although a growing therapeutic armamentarium can only be a positive step, success rates for individual therapies remain ~20-30%.[4] In response to this, there are ongoing drug development efforts to identify new compounds with different mechanisms of action.[4] However, an equally important approach will be to identify molecular biomarkers that can predict response. In this way, patients who require additional treatment could be matched to the drug that is most likely to work. Several clinical tools have been proposed to predict whether a patient will respond to a particular therapy,[61-66] but these are dominated by clinical features indicative of more severe disease rather than any biological difference relating to the therapy (e.g. low albumin, anti-TNF exposure, previous surgery). These clinical parameters capture little / none of the molecular heterogeneity of IBD and lack the specificity for a single compound that would be needed to guide treatment for an individual patient.

*Anti-TNFα*: Despite having been approved for more than 20 years, we are only beginning to understand the biology that determines response or non-response to anti-TNF therapy.[67-74] Initial transcriptomic studies, profiling inflamed colonic tissue of CD and UC patients before anti-TNF therapy, identified 4 genes (*IL13Rα2*, *IL6*, *IL11*, and *TNFAIP6*) which distinguished infliximab responders from non-responders.[67, 68] One of these signals (*IL13Rα2*) was subsequently replicated in independent cohorts,[75, 76] and linked to goblet cell recovery and epithelial restoration after injury.[77] Subsequent

work has focused on whether there might be other pathways causing inflammation in patients who do not respond to anti-TNF. Utilising an elegant experimental design, *West et al.* identified *oncostatin M* (*OSM*), a member of the interleukin-6 family, as one of the most abundantly expressed cytokines in anti-TNF non-responders, and showed that this could lead to intestinal inflammation in a TNF-independent manner.[78] Computational approaches, integrating publicly available data with newly generated data, have also highlighted mucosal *TREM1* as a potential biomarker for anti-TNF response.[70, 79] Moreover, *TREM1* expression in whole blood has been shown to correlate with anti-TNF response, but conflicting data exist on the direction of the *TREM1* signal (up or down-regulation in anti-TNF non-responders)[70-72] meaning that more work is needed. Understanding the biological basis for the association of *TREM1* with anti-TNF response is an important next step, and may relate to the effect of monocyte *TREM1* levels on autophagy and Fc-gamma receptor signaling,[80] processes that have been previously linked to anti-TNF response.[81-85]

Beyond examining the expression of individual genes / proteins, several efforts have been made to characterise the cell populations that might correlate with response to anti-TNF. For example, detailed immunophenotyping of mucosal and blood cells in patients before and during anti-TNF therapy revealed an expansion of apoptosis-resistant intestinal TNFR2+ IL-23R+ T-cells in anti-TNF non-responders.[73] Likewise, intestinal CD4+ T cells from anti-TNF responders have been reported to produce lower amounts of IL-22BP, while still expressing IL-22, suggesting that anti-TNF-α therapy may act – at least in part – by selectively regulating the expression of specific genes in certain cell-types.[86] Finally, single cell sequencing has helped identify a pathogenic cellular module (GIMATS) that is associated with resistance to anti-TNF therapy.[74] Driven by a unique mononuclear phagocyte-dependent cytokine network, the GIMATS module includes increased numbers of IgG plasma cells, inflammatory mononuclear phagocytes and activated T and stromal cells. This result is consistent with several of the key signals identified in bulk transcriptomics, including *OSM*. Whether these novel insights can result in clinically useful biomarkers or new therapeutic targets remains unclear but certainly warrants further investigation. It will also be important to clarify how many of these non-response signals are specific to anti-TNF therapy, or rather reflect a more refractory phenotype, which has recently been proposed for *OSM*.[87]

Other novel approaches to biomarker development are currently being investigated, with confocal imaging among the most promising.[74] In an exploratory study of 25 patients, adalimumab was fluorescently labelled and sprayed onto the most inflamed intestinal areas prior to anti-TNF treatment. Confocal imaging was then used to enumerate cells with membrane-bound TNFα, and allowed patients to be stratified into two groups: those with <20 positive cells had a 15% clinical response rate by week 12, whereas those >20 had a 92% response rate. Although validation in a larger, independent cohort is needed, the idea of quantifying the therapeutic target to estimate the likely response rate is attractive and biologically meaningful.

Whether genetics can help in the prediction of response to anti-TNF is still debated, as none of the proposed genetic markers from small-scale studies have replicated in larger, independent cohorts.[88-90] However, pharmacogenetic studies to identify markers that correlate with specific adverse events have been more successful. For example, the PANTS study (Personalising anti-TNF therapy in Crohn's disease Study), a prospective UK-wide, observational study of 950 patients, reported a robust HLA association with the formation of anti-drug antibodies.[91, 92] Carriage of the HLA-DQA1*05 haplotype was shown to almost double the risk of immunogenicity, and would provide a simple means to guide clinical decisions regarding mono- vs combination therapy in patients who require anti-TNF treatment. The exact 4-digit HLA allele responsible has been debated,[92, 93] but genetic passports that assess HLA-DQA1*05 carriage and other validated treatment-related genetic associations (e.g. for azathioprine toxicity) are already in use in some centres and will likely become more widespread in the future.

*Anti-integrin*: In contrast to anti-TNF agents, vedolizumab's mode-of-action was thought to be well understood with blockade of $\alpha4\beta7$ integrin directly interfering with gut-selective lymphocyte trafficking. However, it is now clear that the biological effect is much broader,[94-97] which in turn makes identifying accurate predictive markers more challenging. For example, there are conflicting reports on the predictive value of $\alpha4\beta7$ expression on circulating T cells.[98, 99] Baseline expression of four genes (*PIWIL1*, *MAATS1* (*CFAP91*), *RGS13*

and *DCHS2*) in inflamed colonic mucosa was reported to predict endoscopic response to vedolizumab, but not anti-TNF therapy, with some promising preliminary validation,[100] although larger studies are required.

In line with its successful application in anti-TNF therapy, enumeration of target cells for anti-integrin therapy has also been investigated. In a small study, the number of α4β7-expressing cells in intestinal biopsies was assessed using imaging and shown to correlate with vedolizumab response: therapeutic benefit was observed in two patients with α4β7-expressing mucosal cells, but not in those patients lacking these cells.[101] Microbial predictors have also been sought in vedolizumab-treated patients. A combination of clinical phenotyping and microbiome data was reported to predict response to vedolizumab in 85 IBD patients.[100] Unfortunately, like many of the predictors described in this review, these have not yet been independently validated.

*Anti-IL12/23p40 and anti-IL23p19, Janus Kinase inhibitors and Sphingosine 1-phosphate modulators*

Multiple new compounds with different mechanisms-of-action have recently been licensed for use in IBD or are in late stage trials. This growing number of therapeutic options will amplify the need for biomarkers that can identify the best treatment for individual patients. Encouragingly, this consideration is now being factored into trial design – for example serum IL-22 was identified as a potential biomarker for brazikumab efficacy (anti-IL23p19) during the phase IIa program.[102] However, it is clear that more research is needed, particularly for comparative biomarkers that will not only estimate a patient's chance of response to a single agent, but will be able to indicate which therapy would be most appropriate. Such efforts are underway, for example the IBD-RESPONSE study is seeking to identify metagenomic signatures that correlate with response to several biologic therapies. Relatedly, there is also a need to better understand some of the predictors that have emerged from several studies. For example, patients with a higher baseline microbial diversity appear to derive better responses to any therapy,[103, 104] as do patients with a lower abundance of pro-inflammatory bacteria, fewer mucus-colonizing bacteria and a higher abundance of short-chain fatty acid-producing bacteria.[103, 104] The exact reasons for this are unknown but may provide therapeutic targets, as well as potential biomarkers.

Finally, there is a growing interest in integrating multiple data sources, including clinical parameters, rather than simply using a single parameter (**Box 3**). Such multi-omic approaches, which may better reflect the complexity of IBD pathogenesis, integrate many different features from the same individual, including gene expression, genetics, microbiome and clinical parameters, and may provide additional insights into disease mechanisms, and/or potential biomarkers.[5] For example, in a paediatric inception cohort of UC patients, the integration of microbiota, mucosal transcriptomics and clinical data was reported to predict need the for anti-TNF treatment escalation.[105, 106] However, while such multi-omic approaches may provide deeper insights into disease heterogeneity,[107, 108] they will in turn create additional challenges in generating a test that can be easily translated for use in routine clinical practice.

## Conclusions and future directions

Considerable research efforts are now being focused on developing prognostic and predictive tools that could make personalised therapy a reality in IBD (**Box 3**). The attrition rate of promising biomarkers in oncology, however, highlights the need for appropriate methodology and careful analysis at every step of biomarker development (**Box 1**).[109] Indeed, there are several key requirements, which should be considered minimum standards in future studies. These include the need for independent validation in well-powered, clinically comparable cohorts that are entirely separate to those used for biomarker discovery. Studies should ideally be prospective, to reduce the chance that a biomarker result is due to a particular disease course or side-effect that has already occurred. This will necessitate using samples taken before a treatment is initiated for treatment-response biomarkers, and samples taken close to diagnosis or immediately post-operatively for prognostic biomarkers (i.e. at a time when intervening with a different treatment strategy would be desirable). More broadly, it will be important to have robust definitions of the endpoints that we would want to predict, and to ensure these are applied consistently across studies wherever possible. The recently published SPIRIT consensus on outcomes for disease modification studies[110] provides a helpful guideline for this, but it is important to recognise that different endpoints will almost certainly be required for prospective and retrospective studies. For example, using

permanent bowel damage, short bowel syndrome or dysplasia as endpoints in prospective studies of newly diagnosed patients would be not be realistic or feasible. Likewise, although retrospective studies could use such endpoints, these are unlikely to have samples taken close to diagnosis and so will lack prospective validation. Clinical practice will have to inform endpoint selection, but variability in IBD management might mean that the same endpoints cannot be used in all cohorts. When appraising biomarkers, it is also important to question whether they are truly predictive or simply represent statistical associations with a given outcome (**Box 1**). Being significantly associated does not mean that a biomarker will be predictive. For example, the well-known association between late adolescence / early adulthood and onset of IBD is highly statistically significant, but this does not mean that being a young adult is predictive of developing Crohn's disease or UC.

Finally, there remains a need to better understand the biological determinants of treatment response and disease course. Some advances in this area have been made,[45] but biomarkers are frequently reported without considering the underlying biological mechanisms. This would not only provide a "sense check" to confirm that the clinical association is biologically plausible, but might also provide new opportunities for treatment. For example, there is growing evidence that the determinants of disease course are different to those involved in disease development,[45, 111] which could provide opportunities to convert a patient with aggressive IBD into one with mild disease – a possibility that remains unexplored.

In summary, technological advances combined with a broader armamentarium of approved therapies have driven a renewed interest in developing biomarkers that could guide treatment decisions for patients with IBD. A major challenge, however, which is common to all biomarker development programs, is the length of time that it takes to develop, validate and commercialise biomarkers – with successful examples often lagging several years behind therapeutics advances.[112] Nonetheless, the current situation in IBD mirrors that seen in oncology a few years ago, and the subsequent successful implementation of personalised cancer therapy provides hope that we will soon have clinically useful tools that can help deliver personalised therapy in IBD.

## Figure legends

**Figure 1 The future of personalised therapy in IBD**
Future implementation of personalised therapy in IBD is likely to require a combination of biomarkers. Prognostic biomarkers will first be required to identify which patients can be safely and appropriately managed using standard treatment strategies and which require more aggressive therapy from diagnosis. Predictive biomarkers (for treatment response and side-effects) will then be required to assign patients to the therapeutic strategy that is most appropriate for them.

**Figure 2 Biomarker performance, risk and uncertainty**
A biomarker is developed to predict response to a new treatment to which 50% of unselected IBD patients will respond. The biomarker identifies 2 subgroups of patients. The first subgroup, containing 40% of all patients, has an 88% response rate, while the second, containing the remaining 60% of patients, has a 25% response rate. The performance characteristics – based on these results – are shown. Despite being an effective biomarker with impressive predictive performance, 20% of patients will be “misclassified”.

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**Box 1: Challenges in biomarker development**

1. Prediction not retrospection: while retrospective studies are easier to perform and lend themselves to larger cohorts and longer-term endpoints, clinically useful biomarkers have to be predictive. This means samples must be taken before the endpoint occurs, and will typically require prospective studies unless the biomarker can be shown to be unaffected by time and disease duration.

2. Prediction not association: there is an important difference between association and prediction. Just because a potential biomarker shows a statistically significant association with a particular phenotype does not mean that this would be predictive. When deciding whether a potential biomarker would be useful clinically, consider the predictive performance (sensitivity, specificity, positive predictive value and negative predictive value) not just whether the association is statistically significant.

3. Appropriate validation: validating potential biomarkers in fully independent cohorts is a critical step to assess their predictive performance. These validation efforts need include enough patients to provide statistical power to detect any reported effect, and involve the same endpoints and patient characteristics as those used in biomarker development. Failure to do this could lead to false negative or false positive results.

4. Communicating uncertainty: even with effective biomarkers, we will not be able to predict the future with 100% certainty. Biomarker results will most likely predict a patient's relative risk of a particular disease course / side-effect / treatment response, and being able to understand and explain this to patients will be an important challenge (**Figure 2**).

**Box 2: Summary of promising biomarkers that have been validated or are undergoing validation prior to clinical implementation**

1. Prognostic biomarkers

    1.1 PredictSURE IBD®: 17 gene whole blood qPCR classifier separating IBD patients with mild and aggressive disease course at diagnosis, validated and currently being tested in the 'Predicting outcomes for Crohn's disease using a molecular biomarker' (PROFILE) trial

    1.2 Prognostic serum protein profile (derived from the IBD character and Swedish Inception Cohort) currently being investigated in the Nordic IBD treatment strategy trial (NORDTREAT)

2. Biomarkers predictive for therapeutic efficacy

2.1. Single gene transcriptomic markers validated across independent cohorts:

- Mucosal IL13RA2 and anti-TNF failure
- Mucosal OSM and anti-TNF failure, being part of the larger GIMATS module
- Mucosal and whole blood TREM1 (conflicting data) and anti-TNF failure

2.2. HLA-DQA1*05 variants and anti-TNF immunogenicity

2.3. Multi-(omic) markers: none have been replicated or are currently undergoing validation in a randomized trial design

3. Biomarkers predictive for adverse events

3.1 HLA-DQA1-HLA-DRB1 variants and thiopurine-induced pancreatitis

3.2 NUDT15 variants and thiopurine-induced myelosuppression

3.3 TPMT variants and thiopurine-induced myelosuppression

**Box 3: Large biomarker studies aiming to unravel disease heterogeneity and improve precision medicine in inflammatory bowel disease**

Multi-omic projects

- Innovative medicines initiative (IMI) 3TR: identification of the molecular mechanisms of non-response to treatments, relapses and remission in autoimmune, inflammatory and allergic conditions
- Innovative medicines initiative (IMI) Immuniverse: exploring the universe of microenvironment-imposed tissue signatures and their correlates in liquid biopsies
- SYSCID – A Systems medicine approach to chronic inflammatory diseases (Horizon 2020)
- RISK cohort (NCT00790543): prospective study of treatment-naïve newly diagnosed pediatric patients with Crohn's disease
- IBD Plexus is an interconnected exchange platform with various purposes, including biomarker identification and hypothesis validation.
- IBD Multiomics database aiming to gain understanding of the complex interplay in IBD
- Precision Medicine in Chronic Inflammation (PMI) aiming to develop molecular tools for treatment of chronic inflammatory disease

Proteomic projects

- Collaborative IBD Biomarker Research Initiative (COLLIBRI) aiming to unravel disease heterogeneity in IBD
- PREDICTS (PRoteomic Evaluation and Discovery in an IBD Cohort of Tri-service Subjects): biorepository study (USA military personnel) aiming to identify novel proteomic biomarkers particularly before development of IBD

Transcriptomic projects

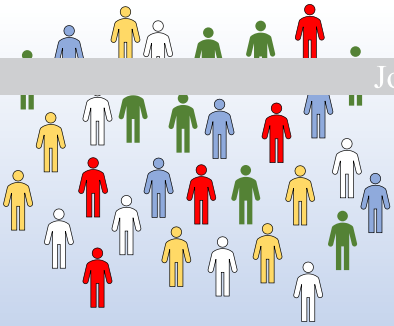
- PROFILE trial (ISRCTN 11808228): biomarker-stratified trial in patients with newly diagnosed Crohn's disease using the PredictSURE IBD

Metabolomic and microbiomic projects

- PREdiCCt (ISRCTN 67248113): observational study investigating how environmental factors, diet and the microbiome influence IBD flare and recovery
- IBD-RESPONSE: prospective study of genetic and metagenomic markers of response to biological and JAKi therapy in IBD.

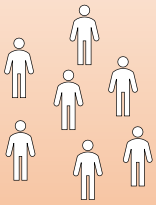
Genetic projects

- PANTS (NCT03088449): observational study aiming to provide novel insights into anti-TNF (non-)response
- IBD Bioresource: observational UK study aiming to further understand the functional effect of IBD-associated gene variants

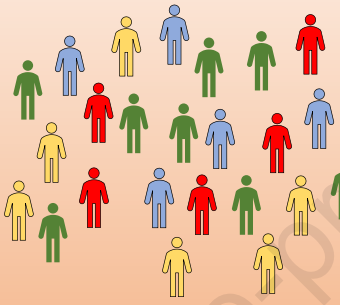


All patients

PROGNOSTIC BIOMARKERS

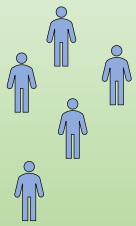


Indolent disease (standard care)

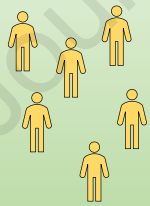


Aggressive disease

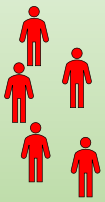
PREDICTIVE BIOMARKERS



Treatment A

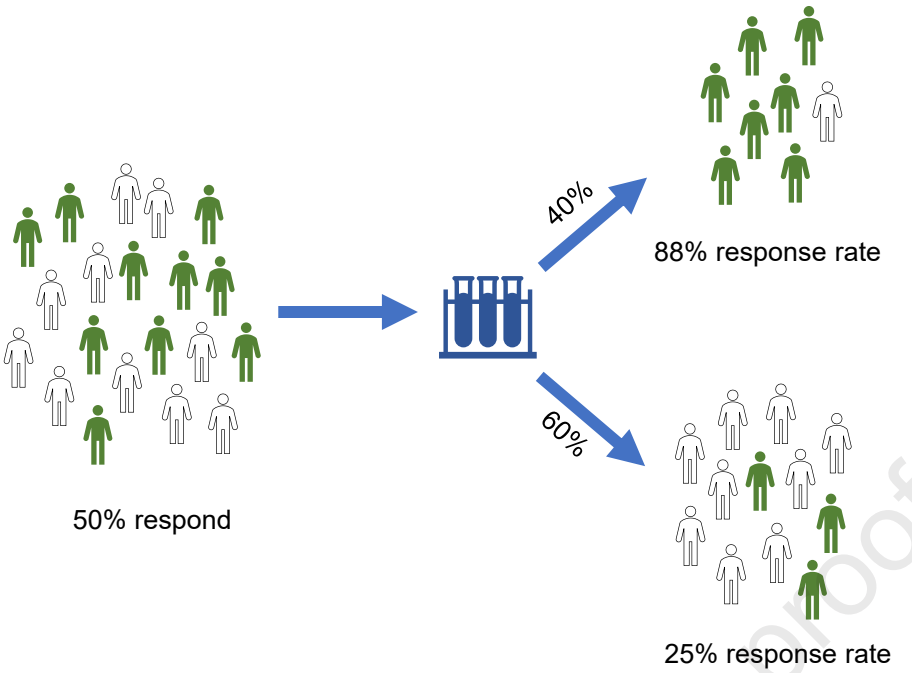


Treatment B



Treatment C

Treatment D




**Performance characteristics**

- Specificity 90%
- Sensitivity 70%
- PPV 88%
- NPV 75%
- Relative risk 3.5

Number “misclassified” 20%