**Open**

Therapeutic Targets in Inflammatory Bowel Disease: Current and Future

Janine Bilsborough, PhD¹, Stephan R. Targan, MD² and Scott B. Snapper, MD, PhD³,4,5

The etiology of inflammatory bowel disease (IBD) is not fully understood but is likely influenced by numerous factors such as genetic predisposition, environmental factors, like the microbiota, and social behaviors, including smoking and diet. In addition to anti-tumor necrosis factor therapy, we now have integrin inhibitors that target lymphocyte trafficking to the gastrointestinal tract (vedolizumab and natalizumab); however, unmet medical needs remain. Although several immunologic pathways have been implicated in the pathogenesis of IBD, targeting these with drug therapy has produced mixed results. Recent drug failures, particularly anti-interleukin-17 (anti-IL17) in Crohn’s disease and anti-IL13 in ulcerative colitis, highlight our fundamental lack of understanding of the impact of pathogenic heterogeneity in the treatment of IBD. In addition to a “failure of mechanism,” other reasons for drug failure may include issues with the drug itself—for example, insufficient activity, or lack of targeting, inadequate trial design or dosing, or potentially our inability to characterize patients who may be more (or less) responsive to a specific therapy. This review summarizes some of the successes and failures and evaluates therapies currently under investigation for IBD.

**IBD ETIOLOGY AND PATHOGENESIS**

Inflammatory bowel disease (IBD) is a collection of disorders characterized by idiopathic inflammation of the gastrointestinal tract. Although primarily classified as either ulcerative colitis (UC) or Crohn’s disease (CD), the presentation of IBD can vary in terms of clinical symptoms, including location of disease involvement, disease severity, age at diagnosis, and presence of extraintestinal manifestations. The etiology of IBD is not fully understood but is likely influenced by numerous factors such as genetic predisposition, environmental factors, like the microbiota, and social behaviors, including smoking and diet. These factors are thought to mediate subsequent epigenetic and immunological changes that contribute to the heterogeneity in the pathogenesis leading to disease. Figure 1 outlines major pathways thought to drive disease pathogenesis and corresponding drug targets. Recent drug failures in IBD, particularly anti-interleukin 17 (anti-IL17) in CD and anti-IL13 in UC, together with the mediocre performance of therapies that target anti-IL12p40, anti-IL23p19, and mucosal addressin cell adhesion molecule (MadCAM), highlight our fundamental lack of understanding of the impact of pathogenic heterogeneity in the treatment of IBD.

Serological, genetic, and mucosal markers can define different clinical subphenotypes within CD and UC, thus implicating diverse biological pathways/mechanisms driving disease within these groups. For example, high levels of anti-neutrophil cytoplasmic antibodies are not only associated with a subpopulation of UC patients (1), but are also associated with nonresponse to anti-tumor necrosis factor (anti-TNF) therapy (2). Antibodies to Saccharomyces cerevisiae are more likely associated with severe disease in CD, defined by fibrostenotic and internal perforating disease, resulting in the need for small-bowel surgery (3). Genetic studies find certain risk loci confer susceptibility to particular subphenotypes of both CD and UC (4,5), and predictive modeling based on a combination of phenotype, serologic, and genetic variables can identify patients who are likely to have a faster progression to surgery (6) and are nonresponsive to anti-TNF (7). These studies not only emphasize the disease diversity in IBD but also define a process of patient stratification that could mitigate the difficulties in treating disease driven by such variable pathogenic mechanisms.

Disease heterogeneity is likely to be a major contributing factor to the frustration of successful drug development and new innovative therapies in recent years. There has been a veritable drought in drug approvals between 1998 and 2014, with vedolizumab, a monoclonal antibody specific for α4β7 integrin, being the most recent non-TNF-targeted drug approved for moderate-to-severe UC and CD (8). Although many new investigational drugs have reached...
clinical trials in recent years, the majority fail to demonstrate sufficient efficacy to justify continued development.

FAILURES IN IBD: LESSONS LEARNED

Cytokine-based therapies

Anti-IL17A (secukinumab). Arguably, the most surprising drug failure for IBD in recent years has been the anti-IL17A monoclonal antibody, secukinumab. IL17A induces numerous inflammatory mediators, including proinflammatory cytokines, chemokines, and matrix metalloproteinases, and plays a role in anti-microbial responses. IL17 is elevated in IBD patient serum, tissue biopsies, and cells isolated from tissues (9,10). The in vitro mechanistic studies strongly implicate IL17A in the pathogenesis of IBD, but no therapeutic effect of IL17A neutralization in CD patients was observed. Indeed, some patients demonstrated acute exacerbation of disease following treatment (11). These data were unexpected as anti-IL17A treatment is efficacious for psoriasis (12) and ankylosing spondylitis (13), both of which are inflammatory diseases that demonstrate significant overlap with IBD in biological pathways and genetic susceptibility associations (14).

Of note, the controversial data from preclinical animal models may have predicted the failure of IL17A blockade in human IBD, and may yet provide insight into the biological role of this cytokine in the gut. IL17A antibody neutralization in dextran sodium sulfate-induced colitis resulted in aggravation of disease, whereas IL17A-deficient mice were resistant (15,16). In T-cell transfer studies, anti-IL17A blockade had no effect on the development of colitis, but administration of IL17A induced disease (17), whereas IL17A-deficient mice were susceptible (18). Although studies in genetically modified mice may confound mechanistic interpretation of the results, these studies suggest a multifaceted role for IL17A in the gut. IL17A has the potential to protect both mice and humans from colitis through regulation of Th1 (T helper type 1 cell) responses, maintenance of barrier function, production of protective IL22, or by supporting anti-microbial activities (reviewed) (19). These data, together with studies showing the presence of Foxp3+IL17+ CD4 cells with immunosuppressive activity
in colitis patients (20) and the generation of protective Th17 cells through effector cell plasticity in murine colitis models (21), highlight potential reasons for failure of IL17 blockade in IBD. The lack of understanding the intricacies of IL17 biology highlights the difficulties of comprehending how multifactorial components contribute to the regulation of IBD pathobiology and influence our ability to modify the disease course.

**Anti-IL13 (tralokinumab and anrukinzumab).** In the face of compelling preclinical data, the failure of anti-IL13 therapies in UC was disappointing. Increased production of IL5 and IL13 by T cells, natural killer cells and natural killer T cells isolated from UC patients provided support for the current dogma that IL13 is a driving factor in UC pathogenesis (22–24). However neither tralokinumab, a fully human anti-IL13 monoclonal antibody, nor anrukinzumab, a humanized anti-IL13 antibody that blocks IL13 binding to IL4Rα, induced positive results in recent phase IIa clinical trials for moderate-to-severe or mild-to-moderate UC (25,26).

The design of the anrukinzumab trial, which included an ex vivo assay confirming the biological activity of drug levels in serum of treated patients, left little doubt as to the lack of efficacy in UC. Studies in asthma trials with an anti-IL13 antibody have suggested that patients with high periostin levels may respond better to treatment (27). It remains to be determined whether there are a subset of periostin-high UC patients who could be successfully treated with anti-IL13 therapies. Other factors potentially contributing to the failure of anti-IL13 therapy include the role of IL13 in the development of alternatively activated macrophages that are important in wound healing and, upon transfer, can inhibit colitis in mice (28).

**IL10.** The regulatory role of IL10 in intestinal inflammation was reinforced by the generation of IL10-deficient animals that, when housed under non-germ-free conditions, developed spontaneous chronic enterocolitis (29). Genetic studies are striking in their associations with IL10 dysregulation and the development of IBD. IL10 has been identified in international genome-wide association study (GWAS) analysis as being associated with IBD and UC, as have several signaling molecules that are downstream of IL10-dependent signals (e.g., Tyk2; Stat3) (14). Moreover, patients with loss-of-function mutations in IL10 or the IL10 receptor show early onset of disease (30–33). Early treatment with allogeneic hematopoietic stem cell transplantation is the current recommended treatment for these individuals (34).

Nonetheless subsequent clinical trials assessing IL10 dosing in CD or ileal and ileocolonic postoperative occurrence were discouraging (reviewed) (35). Speculation as to why this strategy failed includes: poor stability, dose limitation at tissue sites, limitations in the ability of IL10 to reverse established disease, limitations of IL10 as a global immunosuppressor of a wide variety of immunostimulators, and the finding that IL10 can also induce proinflammatory cytokines under certain conditions by certain cell types (36,37). Although novel mechanisms for delivering IL10 to tissues are actively being investigated, including the use of oral biodegradable microspheres, microbial delivery systems, and IL10 fusion molecules that target inflamed tissue sites (38–41), concerns remain as to the ability of IL10 to control chronic disease.

**Anti-trafficking therapies**

The infiltration and retention of leukocytes in tissues is a hallmark of inflammation. Both CD and UC are characterized by inflammatory cell infiltrates, although the two diseases differ in the extent and type of infiltrate (42). With the success of natalizumab (anti-α4 integrin) and, more recently, vedolizumab (anti-α4β7 integrin) in the treatment of IBD, anti-trafficking therapies are a popular strategy for future therapeutics.

**MAdCAM-1 (PF-00547659).** MAdCAM-1 is expressed by intestinal endothelium and upregulated in IBD (43). Whereas vedolizumab binds α4β7 on lymphocytes, anti-MAdCAM-1 antibodies bind their target on endothelium, blocking leukocyte adhesion, and thus preventing lymphocyte entry into gut tissue (44). PF-00547659 is a fully human monoclonal antibody that has been tested for efficacy in both CD and UC clinical trials (45).

Blockade of MAdCAM-1 in CD patients with active moderate-to-severe disease and a history of failure to anti-TNF (OPERA) was assessed using a drop in CDAI (Crohn's Disease Activity Index) as the primary end point. No significant improvement in CDAI-70 was observed at week 12, although pharmacological activity of the drug was confirmed through observed dose-dependent decreases in soluble MAdCAM levels and increases in circulating T cells (46).

In contrast, significant clinical remission was achieved with anti-MAdCAM therapy in the UC trial (TURANDOT). Moreover, the secondary end point of mucosal healing was also significantly improved in the drug treatment groups. In addition, greater efficacy was observed in anti-TNF-naïve patients compared with patients previously treated with anti-TNF agents (47).

The results above suggest that strategies that aim to block leukocyte recruitment generally appear more successful in UC than CD. The reasons for this are not understood but may reflect differences in the intestinal compartments associated with both diseases. T cells are less prominent in tissues of UC compared with CD, with inflammation in UC limited to the mucosa and submucosa, whereas inflammatory cells are present throughout the gut wall in CD (reviewed in Macdonald and Monteleone (48)). Thus, inhibition of leukocyte recruitment in UC may be more obtainable, resulting in better efficacy with anti-trafficking therapies. Indeed, anti-addressin therapy may simply require longer treatment periods in CD compared with UC to achieve a similar clinical response. Alternative theories to explain the differences in efficacy relate to a potential dominant effect of different leukocyte recruitment molecules within the different tissue compartments and/or the different inflammatory environments of UC vs. CD. CCR9, for example, is specific to the small bowel (49) and it is conceivable that a novel mechanism involving adhesion/trafficking molecules in the colon may contribute to the superior efficacy of existing anti-trafficking strategies in UC.

Such differences in efficacy between CD and UC clinical trials for MadCAM blockade echo those observed with vedolizumab.
Although vedolizumab has been approved for both CD and UC, the results from the UC clinical trial was more robust (50–53). Similarly, the observation that MAdCAM blockade is more efficacious in patients not previously treated with anti-TNFs hints at important insights into the biological mechanisms relevant to the context in which these drugs are delivered. Studies observing downregulation of integrins following anti-TNF therapy raised concerns that prior exposure of anti-TNF could potentially mitigate efficacy of integrin-targeted therapies (54). However, a recent meta-analysis of α4 integrin clinical trials suggested no significant difference in efficacy between anti-TNF-naive and exposed patients (55). A clinical trial aimed at studying the impact of prior exposure of anti-TNF on vedolizumab effectiveness is currently planned and will hopefully add further clarity to this issue (ClinicalTrials.gov: NCT02423512).

**Anti-ICAM (alicaforsen).** Intercellular adhesion molecule-1 (ICAM-1) is expressed on dendritic cells, fibroblasts, epithelial cells, and endothelial cells (56). ICAM-1 is upregulated in the presence of proinflammatory cytokines and downregulated following treatment with anti-TNF (57). Alicaforsen is an ICAM-1 antisense oligonucleotide that specifically blocks ICAM-1 mRNA and subsequent protein expression. Consistent with other leukocyte trafficking strategies, ICAM-1 blockade shows greater efficacy in UC than CD (58,59), although a contributing factor may be that local delivery of the antisense oligonucleotide is required to achieve better clinical results (60).

### CHALLENGES IN IBD: THE STRUGGLE FOR SUCCESS

#### Cytokine-based therapies

**Anti-IL12p40 (anti-IL12 and anti-IL23 blockade).** IL12p40 is a cytokine subunit of both IL12 and IL23, two cytokines that have been implicated in the pathogenesis of IBD (61). Whereas IL23 is an important cytokine for the maintenance of Th17 cells (62), IL12 is essential in the development of Th1-type responses, resulting in increased interferon-γ and enhanced cell-mediated immunity. GWASs indicate that variants within the *IL12p40* locus confer genetic risk for the development of IBD (14).

Early clinical trials with an anti-IL12p40 antibody, ustekinumab, in CD patients demonstrated a significant clinical response within 4–6 weeks of treatment. Moreover, in patients who were previously treated with anti-TNF, the response rate to ustekinumab was more striking, although this was largely because of lower placebo rates in anti-TNF-exposed patients (63). Subsequent trials in patients refractory to anti-TNF therapy supported the notion that these patients were more likely to respond to ustekinumab (64). In contrast, briakinumab, also a human monoclonal antibody against anti-IL12p40 generated by phage display technology, did not meet the primary end point of clinical remission at 6 weeks, nor were response rates significant (65).

A meta-analysis designed to compare the efficacy of ustekinumab and briakinumab concluded moderate efficacy of ustekinumab in inducing a clinical response (66). The reason for the differences in efficacy between ustekinumab and briakinumab are not entirely understood. Comparative analysis of the half-lives of the two antibodies suggests that briakinumab has a shorter half-life than ustekinumab (67) and may also have lower affinity (patents US 8,765,918 B12 and US 6,902,734 B2), although both of these issues can be overcome by an adequate dosing regimen. Nevertheless, these data suggest that blockade of both IL12 and IL23 only moderately affected disease in CD patients. Results for the latest phase III clinical trial in CD are expected to show better efficacy because of increased dose (www.clinicaltrials.gov). Despite the genetic association of IL12p40 and IL23R with both CD and UC (14), clinical trials testing anti-IL12p40 therapeutic efficacy in UC have not been initiated.

**Anti-IL23p19.** Numerous GWASs have identified genetic variants in the IL23R loci as conferring susceptibility toward IBD in diverse ethnic groups (68–72). Moreover, studies assessing T cells from donors carrying the protective IL23R R381Q polymorphism (71,73) suggested impaired IL23-dependent signaling transducer and activator of transcription (STAT) activation (74), thus providing mechanistic evidence for the observed protective association. The pathological role of IL23 in IBD is also supported by evidence of enhanced production in both CD and UC patients (61). Preclinical studies using IL23R-deficient mice in the T-cell transfer model of colitis suggest IL23 favors the emergence of pathogenic IL17/interferon-γ-positive cells in the colon and downregulates FoxP3-positive cells (75).

Recent clinical trial data for anti-IL23p19 blockade (MED2070) in CD patients who have failed anti-TNF therapy demonstrated a significant clinical effect at week 8, as defined by ≥50% reduction in fecal calprotectin (76). However, as reported in IL12/IL23 blockade trials, there was a failure to meet clinical remission end points. The disconnect between reduction in fecal calprotectin and clinical remission may reflect the need for a longer duration of treatment to observe changes in clinical end points.

**JAK inhibitors (tofacitinib).** Janus kinase (JAK) inhibitors are small-molecule therapies that target the nonreceptor tyrosine kinase family. This family of proteins associates with the intracellular domain of cytokine or hormone receptors to facilitate signal transduction. JAKs phosphorylate tyrosine residues on the cytokine receptor, creating a docking station for a group of transcription factors known as STATs. STATs are subsequently phosphorylated by JAKs that leads to STAT dimerization, nucleus translocation, and transcriptional activation of target genes (77).

GWASs have identified single-nucleotide polymorphisms within the JAK/STAT pathway that confer susceptibility to IBD (14), and both in vivo and in vitro studies have confirmed the critical role of JAK/STAT signaling in regulating immune responses (78). Tofacitinib, an inhibitor of JAK1, JAK2, and JAK3, has been assessed in clinical trials for both UC (79) and CD (80). In UC, the highest dose (15 mg) achieved significant clinical response and remission rates as well as endoscopic response and endoscopic remission rates (79). In contrast, in CD, no significant difference in clinical remission or clinical response was observed with tofacitinib (maximum dose 15 mg), although reductions in C-reactive protein levels (79)
and fecal calprotectin levels were observed, suggesting biological activity of the drug (80). Reasons for the differences in efficacy between CD and UC are not understood. It may be that higher doses or longer treatment regimens are required for CD, although adverse effects on blood lipids will likely limit dosing in future clinical trials.

**Augmenting TGFβ (SMAD7-inhibitor: mongersen).** Transforming growth factor-β (TGFβ) is a multifunctional protein that regulates many cell processes including proliferation, differentiation, adhesion, and migration. This cytokine has long been implicated in immunosuppression of cellular responses and in murine models; administration of TGFβ can abrogate the development of colitis, whereas blockade can exacerbate disease (81–83).

TGFβ1 modulates its effects through type I and II receptor serine/threonine kinases resulting in phosphorylation of the kinase domain of the type I receptor and subsequent phosphorylation of receptor-regulated SMAD proteins. The activated SMAD complexes translocate to the nucleus where they regulate transcription of target genes (84). There are three functional classes of the SMAD proteins: receptor-regulated SMADs that facilitate gene transcription, the co-repressor SMADs, and the inhibitory SMADs. SMAD6 and SMAD7 lie within the class of inhibitory SMADs. These down-regulate TGFβ signaling by competing with the receptor-regulated and co-repressor SMADs for binding to the phosphorylated type I receptor, and by targeting the type I receptor for degradation.

SMAD7 is upregulated in both UC and CD tissues (85). Decreased ubiquitination and subsequent degradation is suggested as the primary cause for elevated levels (86). Preclinical models support inhibition of SMAD7 through antisense delivery (87) and a subsequent phase II trial in CD patients has shown promising results. Of the patients, 65% met the primary end point of clinical remission (vs. 10% of placebo), whereas 72% of patients demonstrated a clinical response at day 28 (vs. 17% of placebo) (88). However, no significant reduction in C-reactive protein was observed, raising concerns as to the biological effect of this treatment. Nevertheless, assessment of patient-reported outcomes, a composite score of stool frequency and abdominal pain that have been shown to correlate with CDAI (89), confirmed higher clinical response and remission rates in patients receiving the highest doses of mongersen (90). There is no explanation for the disconnect between C-reactive protein level and clinical remission/response, but this does suggest that any future trials must be designed to include multiple measurements of inflammation, including C-reactive protein, calprotectin, and endoscopy as critical endpoints to ensure clear understanding of the benefits of this therapy.

TGFβ1 is also a major driver in the development of fibrosis, through stimulation of mesenchymal cells that results in proliferation and production of extracellular matrix. Thus, concerns were raised regarding a potential increased incidence of stricture disease following prolonged treatment in CD patients. A small phase I open-label trial monitoring the incidence of small bowel strictures and circulating levels of basic fibroblast growth factor, YKL-40, matrix metalloproteinases, and TIMP1 in 15 patients showed no association with the development of small bowel strictures within 180 days of a 1-week daily dosing regimen (91). Because of the small number of subjects and the short duration of these studies, the question regarding the development of stricture disease as a result from prolonged treatment remains open.

**Anti-trafficking therapies**

**SIP inhibitors.** The latest anti-trafficking therapy in the clinic for UC is RPC1063, an oral, selective sphingosine 1-phosphate (SIP) 1 and 5 receptor modulator. SIP is a bioactive sphingolipid metabolite formed by the activity of sphingosine kinases. SIP regulates numerous cellular activities including cell growth, survival, vascular integrity, and lymphocyte trafficking. SIP and SIPR are essential for lymphocyte egress from peripheral lymphoid tissue and thymus; with high levels of SIP in the blood and lymph forming a gradient from lymphoid tissue, promoting the egress of SIPR-positive cells. SIPR agonists block lymphocytes egress by disrupting the SIP gradient or desensitizing the receptor (92). SIPR agonists have been studied in preclinical models of colitis (93–96) and SIP levels are elevated in the blood of UC patients (96).

In an 8-week induction trial for moderate-to-severe UC, 16.4% of patients receiving the highest dose of RPC1063 reached the primary end point of clinical remission at week 8 (vs. 6.2% of placebo, P=0.0482). The proportion of patients with a clinical response was 58.2% for the highest dose (vs. 36.9% of placebo, P=0.0140) and the proportion of patients with mucosal improvement was 34.3% (vs. 12.3% of placebo, P=0.0023) (97). These data are likely sufficient to warrant a follow-up phase III trial in UC.

**Augmenting innate immunity**

The identification of NOD2 as a gene associated with CD brought considerable attention to the conception of innate immune function as an early event in the initiation of CD (98,99). A burgeoning literature has identified impaired innate immunity, not only associated with NOD2, but also including defective barrier function (100), neutrophil function (101), macrophage function (102), reduced dendritic cell function (103), and reduced autophagy (104,105) and paneth cell function among others innate immune abnormalities associated with IBD. The cause of this reduced innate immunity is unclear but may result from genetic impairments, influence of the microbiome, and other epigenetic effects. Therapeutics aimed at boosting innate immune function, without the concomitant associated side effect of immunosuppression, will undoubtedly be a major focus of study and drug development in the ensuing few years.

**GM-CSF.** In this regard, the development of granulocyte macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF) for the treatment of CD was predicated on an early hypothesis that IBD may result from immunodeficiency and, in particular, defective innate immunity (106). The actions of GM-CSF have broad effects including enhancing neutrophil, macrophage, and dendritic cell function, as well as increasing regulatory T-cell function (107). GM-CSF
heavily targets receptors broadly expressed at high levels in the intestinal mucosa; G-CSF acts more exclusively on the neutrophil. After open-labeled trials of G-CSF and GM-CSF demonstrated safety and efficacy for active CD, a randomized controlled phase 2 study for active disease and a placebo-controlled study for use as a steroid-sparing agent both demonstrated efficacy (108–110). A phase 3 study failed to show a benefit but placebo responses were high, and in subanalyses where countries with previously low indices of CD and high placebo rates (>50%) were removed (e.g., Russia, Ukraine, Brazil), the benefit in the remaining countries was statistically significant (111)—hence, some consider this a poorly designed study and that further investigation on this agent may be warranted. This agent may be reformulated for clinical trials in the near future.

FUTURE THERAPIES: WHERE TO NOW?

Clearly, moderate clinical responses and high placebo rates plague IBD drug development. Disease heterogeneity, reflected by variable IBD clinical phenotypes and differences in natural history, likely contribute to the difficulties of achieving high response rates within heterogeneous patient populations. Although understanding the complex biological processes involved in disease pathogenesis is important for target selection, understanding the need for patient stratification in the face of complex confounding factors is just as likely to be a key for success of new therapies.

Genetic studies in IBD confirm the complexity of the disease, extending the number of IBD susceptibility loci to 163 in all (14). Genetics are also associated with disease phenotypes and natural history; for example, NOD2 and TNFSF15 variants are associated with the need for surgery, whereas JAK2 variants are associated with stenosing disease behavior (4). Similarly, serologies can be used to define clinical subphenotypes within either CD or UC, implying differential responses to bacterial antigens in patient subgroups. As genetics have also recently been implicated in influencing the structure of the microbiome (112), it is clear that the host genetic background, together with other environmental factors, could influence the dominant biological pathway that leads to disease pathogenesis. Therefore, stratification through serology, genetics, and clinical phenotype is expected to yield increasingly homogenous patient groups that are predicted to lower the number of patients needed to treat to achieve convincingly positive outcomes in clinical trials.

However, patient selection is not the only issue associated with failed therapeutics. Table 1 gives examples of failed therapeutics and a hypothesis as to the mechanism of failure. Almost all of the examples given have demonstrated differential expression in diseased vs. nondiseased samples. Indeed upregulated target expression in patient vs. healthy samples is often a major prerequisite for target validation, yet there is limited quantitative evidence of increased TNF in IBD vs. non-IBD tissues. The failure of therapeutics such as anti-IL17 in CD and anti-IL13 in UC, both of which are upregulated in the respective diseases, caution against tissue expression as a major criteria for target validation. Tissue expression may not reflect the dominant pathway for disease pathogenesis and does not discern between cause and consequence. Another common theme among the failed therapeutics is the potential for divergent function, i.e., a number of these targets are involved in both induction and resolution of inflammation depending upon environment milieu; Redundant: other factors with similar function will compensate; Dosing: higher dosing or bioavailability may be required or stability of therapeutic is an issue; Regional: tissue pathophysiology differs between small and large bowel; Incidental: target is not essential to pathophysiology as it is downstream of causal event; End point: clinical trial end point was not optimal; Prior exposure: prior therapeutic exposure modifies biology.

<table>
<thead>
<tr>
<th>Targeted mechanism</th>
<th>Example of therapeutic</th>
<th>Efficacy in CD</th>
<th>Efficacy in UC</th>
<th>Potential mechanisms of failure</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Th1 pathway</td>
<td>Anti-IFNγ</td>
<td>No</td>
<td>—</td>
<td>Population; end point</td>
<td>Personal communication</td>
</tr>
<tr>
<td>Th17 pathway</td>
<td>Anti-IL17A</td>
<td>No</td>
<td>—</td>
<td>Divergent function</td>
<td>(19–21)</td>
</tr>
<tr>
<td>Th2 pathway</td>
<td>Anti-IL13</td>
<td>—</td>
<td>No</td>
<td>Redundant; population, incidental</td>
<td>(27,28)</td>
</tr>
<tr>
<td>Lymphocyte trafficking</td>
<td>Anti-MadCAM</td>
<td>No</td>
<td>Yes</td>
<td>Regional; prior</td>
<td>(41)</td>
</tr>
<tr>
<td>Anti-β2</td>
<td>ICAM-1 antisense</td>
<td>&lt;UC</td>
<td>Yes</td>
<td>Exposure; dosing</td>
<td>(58–60)</td>
</tr>
<tr>
<td>JAK/STAT pathway</td>
<td>JAK1/JAK2/JAK3</td>
<td>No</td>
<td>Yes</td>
<td>Regional; dosing</td>
<td>(71)</td>
</tr>
<tr>
<td>Cheemoattrantciant</td>
<td>Anti-leukotrienes</td>
<td>—</td>
<td>No</td>
<td>Redundant, incidental</td>
<td></td>
</tr>
<tr>
<td>Anti-CCR9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL10 pathway</td>
<td>Recombinant IL10</td>
<td>No</td>
<td>—</td>
<td>Dosing; divergent function</td>
<td>(31–34)</td>
</tr>
<tr>
<td>IL6 pathway</td>
<td>Anti-IL6R</td>
<td>?/No</td>
<td>—</td>
<td>Divergent function</td>
<td></td>
</tr>
</tbody>
</table>

CD, Crohn’s disease; IBD, inflammatory bowel disease; ICAM-1, intercellular adhesion molecule 1; IFNγ, interferon-γ; IL, interleukin; JAK, Janus kinase; MadCAM, mucosal addressin cell adhesion molecule; STAT, signaling transducer and activator of transcription; Th, T helper; UC, ulcerative colitis; Th1, Th17, Th2, Th1/Th17, Th1/Th2, Th2/Th17; ICAM, ICAM-1; JAK, JAK1/JAK2/JAK3; MadCAM, MadCAM-1; STAT, STAT1/STAT3/STAT5; Th, T helper; UC, ulcerative colitis.
risk of IBD in GWASs. These factors highlight the need for a well-rounded discovery and validation program that begins with analysis of targets in human disease and includes insight into genetics, clinical pathophysiology, and biological function in the context of environmental factors including the microbiome.

**Novel cytokine-based therapies**

**Anti-TL1A antibodies (TNFSF15).** GWASs have found TNFSF15 variants associated with IBD in European, Japanese, and Korean populations (113–116). In addition, analysis of genetic variants associated with IBD clinical subphenotypes identifies the TNFSF15 locus as a major risk factor for stricturing disease and surgery (4,116). Pre-clinical animal studies in both chronic dextran sodium sulfate-induced colitis and T-cell transfer models have confirmed the link between TL1A (TNFSF15) overexpression and the development of colitis with fibrostenosing disease (117–119). Moreover, TL1A therapeutic blockade can successfully reduce colitis and reverse fibrosis in these models (120). Preliminary *in vitro* studies in humans suggest TL1A is overproduced in patients who carry the risk allele vs. those individuals who carry the wild-type TL1A allele (unpublished observations). Correct patient selection will therefore be key in correctly assessing efficacy of an anti-TL1A therapeutic in CD patients.

**Anti-IL6/anti-IL6R/gp130-Fc.** IL6 is a proinflammatory cytokine that is upregulated in patients with CD (121,122). IL6, in combination with TGFβ, promotes Th17 cell differentiation—cells that are enriched in CD. In classical signaling, IL6 binds to an IL6 receptor/gp130 complex on the cell surface and induces receptor-associated kinases (JAK1, JAK2, Tyk2) that lead to downstream STAT1 and STAT3 signaling events (123). Alternatively, by trans-signaling, IL6 can bind to soluble IL6R and can then activate cells that express gp130 on the cell surface. Although IL6 is well known for its lymphocyte signaling properties, it also controls a variety of homeostatic functions including control of endocrine and metabolic properties. In this regard, blockade of IL6 through antibodies targeting the cytokine or its receptor IL6R will lead to blockade of both lymphocyte-specific effects and more general effects mediated by classical signaling. In contrast, blockade of trans-signaling has been accomplished through the use of a gp130-Fc fusion molecule and has been suggested as an alternative approach use in IBD (123).

A number of clinical studies have employed anti-IL6 or anti-IL6R antibodies to assess blockade of IL6 or IL6R blockade in several autoimmune conditions with efficacy demonstrated for rheumatoid arthritis. Preliminary studies targeting IL6R have suggested some efficacy in CD (124). Phase II studies with anti-IL6 monoclonal antibodies in patients with active CD are underway. Particularly appealing is the approach of blocking trans-signaling selectively with a gp130-FC fusion protein. This approach has the advantage of permitting some of the homeostatic signals through the classical pathway that may be beneficial while blocking trans-signaling-induced events that may be responsible for unwanted inflammatory processes (125). If complete blockade of anti-IL6 signaling is less effective in human studies when compared with murine IBD model, it may be that homeostatic IL6-dependent signals may play a more central role in humans.

**Cellular therapies**

**Autologous/allogeneic hematopoietic stem cells.** Given the number of immune-associated abnormalities associated with IBD and concomitant alterations in the number of immune cell populations and immune-associated cytokines (both proinflammatory and anti-inflammatory) coupled with the number of immune-associated genetic loci identified by large GWASs that modulate IBD risk, it is not surprising that a number of therapeutic approaches employing immune cellular therapies have been developed with the goal of resetting immune balance (126). These include the use of both autologous and allogeneic hematopoietic stem cell preparations in carefully selected patients who have failed standard therapeutic approaches. Autologous stem cell therapy approaches, although only providing “resetting” of an individuals’ immune baseline, have the advantage of not introducing cells with major histocompatibility complex mismatches; in contrast, allogeneic stem cell transplantation offers a new immune system but are associated with increased side effects including graft-vs.-host disease.

To date, autologous stem cell transplantation efforts in CD have been performed in a number of small largely uncontrolled studies. Long-term clinical remission has been reported in a significant number of treated patients. In most of these studies where myeloablative conditioning was performed it has been questioned whether the therapeutic efficacy was due to the conditioning or the transplanted cells. In a phase 1 study with autologous transplantation in the setting of nonmyeloablative conditioning, significant efficacy was again appreciated (127). Severe side effects after transplantation was not uncommon. Small studies have begun to address the role of allogeneic transplantation in CD patients mostly in the setting of underlying hematopoietic malignancy (128). Clinical efficacy has been encouraging—but balanced by the predicted serious side effects. Early open-label phase 1/2 studies are underway evaluating nonmyeloablative conditioning and allogeneic transplantation in the setting of refractory CD (ClinicalTrials.gov: NCT01570348 and NCT01288053). With advanced molecular techniques, identifying the ideal patient as well as donor population will likely be the challenge in the future. Patients with well-defined genetic immunologic defects (e.g., IL10R deficiency; XIAP deficiency) presenting with IBD typically in early childhood are a patient population where hematopoietic stem cell transplantation is particularly effective and increasingly standard of care (129).

**Regulatory T cells (Tregs).** Regulatory T cells (Tregs) are an immune modulating subset of CD4+ T lymphocytes that suppress the activation and effector function of multiple immune cell types that are associated with intestinal inflammation. Humans with mutations in genes that impair Treg function (e.g., FOXP3, IL10R, WASP) are at an increased risk of autoimmunity and intestinal inflammation. As Treg cell therapy has shown promise in other immune-mediated disorders including graft vs. host disease and type 1 diabetes (130), Treg cellular therapies or approaches for the
modulation of Treg numbers in vivo are being developed for IBD (131). A phase 1 study in CD with autologous Tregs has recently been reported as safe and with some efficacy (132). Approaches to expand efficiently autologous Tregs that maintain suppressive function are under development (133). Larger phase 1/2 studies are underway investigating other approaches at expanding Tregs in vivo with exogenous cytokines and biologics (e.g., low-dose IL2; ClinicalTrials.gov: NCT02200445 and European low-dose IL2 study: http://www.iltopharma.fr/?g=node/3, and oral anti-CD3; ClinicalTrials.gov: NCT01287195).

Mesenchymal stem cells. Mesenchymal stem cells are nonhematopoietic stromal cell precursors that have nonimmunogeneic and anti-inflammatory properties that are attained without the need for conditioning. Mesenchymal stem cells have been evaluated in small clinical trials for CD with fistulizing disease or CD/UC patients with luminal disease (126,134). Recently, an open-label phase II study of mesenchymal stem cells in TNF-refractory CD patients demonstrated substantial efficacy with minimal side effects (133). The long-term safety, efficacy, and pathophysiological mechanisms underlying success of this therapeutic modality will await larger studies and deeper scientific investigation.

Targeting the microbiome

With the recent knowledge that IBD is associated with a profound microbial dysbiosis, numerous strategies are under development to deplete microbial constituents that may be pathogenic or to enhance beneficial microorganisms (135). Clarity remains lacking on whether such microbial differences are actually a driving force of intestinal inflammation or a consequence of the proinflammatory milieu. Nonetheless, this dysbiosis has been observed even at the time of diagnosis in untreated patients (136). Despite this paucity of knowledge, certain microbial species have been found to bloom or contract associated with the inflammatory state of IBD. As one representative example, an uncharacterized species of Clostridium cluster XIVa was found to be underrepresented in patients with CD (137). Clostridium species that fall within clusters IV, XIVa, and XVIII isolated from both mice and humans can induce the development of colonic Tregs and suppress inflammation (138,139). In this regard, certain bacterial metabolites such as short-chain fatty acids have the ability to induce Tregs (140) and these metabolites are increased in the presence of dietary fiber (141). Intense investigation has recently focused on clinical approaches to increase such beneficial bacteria or their products employing direct microbial-based treatment strategies, or agents that increase the number or anti-inflammatory activity of such organisms. Strategies to enhance immunoregulatory bacteria or their products or suppress putative deleterious commensal bacteria/metabolites are certain to lead to a new class of microbial-based therapies in the near future.

Financial support: This work was supported by an educational grant from Takeda Pharmaceuticals International, US Region. The organization, writing, and revision of the manuscript was independent of the sponsor.

Potential competing interests: S.B.S. is a consultant or on the advisory board for Pfizer, Merck, Hoffman La Roche, Janssen, Synlogic, Enterome, and Lycera; holds equity in Synlogic, and research grants from Pfizer and Merck. S.R.T. is a consultant for Amgen, Lycera, Janssen, Biogen Idec, Simon Kucher & Partners, and NuMedii, and is on the advisory board for Symbiotix and the Seaver Foundation for Autism.

REFERENCES


