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Future Therapeutic Approaches for Inflammatory Bowel Diseases

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Abstract

In this review, we speculate about future therapeutic approaches for inflammatory bowel diseases (IBDs), focusing on the need for better preclinical and clinical models and approaches beyond small molecules and systemically administered biologics. We offer ideas to change clinical trial programs and to use immunologic and genetic biomarkers to personalize medicine. We attempt to reconcile past therapeutic successes and failures to improve future approaches. Some of our ideas might be provocative, but we hope that the examples we provide will stimulate discussion about what will advance the field of IBD therapy.

Keywords

IBD Therapy; Biomarkers; IBD Immunology

Since infliximab was approved by the US Food and Drug Administration (FDA) for treatment of IBDs in 1998, the identification of new therapeutic targets and the design of clinical trials to evaluate them have become increasingly complex. To speculate about future therapeutic targets and strategies, it is important to review the past 15 years of immunologic interventions for IBD. We propose approaches to developing therapies for IBD, acknowledging that discovery of new information could change our concepts.

In the early 1990s, inhibition of tumor necrosis factor (TNF), a cytokine involved in host defense against pathogenic bacteria and tumors, was not considered to be an effective therapeutic approach for Crohn's disease (CD), and there were concerns about unacceptable adverse effects. An editorial published in 1992[1] about TNF blockade stated “since inflammatory bowel disease is a process that involves several agonists, only drugs which affect most or all of the agonists are likely to be of proven benefit. Drugs which affect one

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single mediator and/or block one single receptor are unlikely to be helpful." Although this conclusion was found to be inaccurate, it was supported by findings from basic and translational studies, reminding us that animal models and biomarkers do not always accurately predict response to treatment.

Studies of levels of inflammatory cytokines measured in blood, intestinal tissue, and stool from patients with IBD have produced conflicting results, and measurements of TNF levels vary.[2,3] There is heterogeneity among patients, and across studies, in levels of cytokines measured in patients with IBD. It is not clear whether this heterogeneity results from technical issues (measurement methods, timing, meals, circadian rhythms, and others), differences among subgroups in the study populations, or both.

Animal models of colitis are useful for addressing mechanistic questions about immune responses; however, their utility in predicting therapeutic responses in people is imperfect. Confusion about the concept of TNF inhibition in CD arose from mixed results of TNF inhibition from studies of different mouse models of IBD. In one model, TNF blockade only prevented colitis[4]; whereas, in another model, anti-TNF agents suppressed active colitis[5]; in some models, TNF blockade does not affect colitis.[4]

Studies of interleukin (IL)-10 in animal models of IBD also failed to predict its effects in humans. IL-10 has pleiotropic, anti-inflammatory effects including suppression of activated T-cell proliferation and inhibition of proinflammatory cytokine production by many cell types.[6] IL-10 prevents onset and reduces active colitis in mice; the occurrence of spontaneous colitis in IL-10-deficient and IL-10 receptor-deficient mice indicated that IL-10 was required for development of colitis.[7] However, IL-10 was not effective in clinical trials of patients with IBD.[8,9] Reasons for failure might include clinical heterogeneity, inability to identify the patient population most likely to benefit, differences in systemic vs local administration (systemic administration might, paradoxically, have proinflammatory effects at high doses[10]), or IL-10 receptor signaling defects in patients with IBD that would make IL-10 replacement ineffective.[11]

Ultimately, identification of therapeutic targets requires a better understanding of why TNF inhibitors are effective in patients with IBD and IL-10 is not. If a rational immunologic model for IBD pathogenesis exists, it must be tested in patients.

Although progress has been made in elucidating the immunologic and genetic features of IBD, there are few biomarkers that predict responses in subgroups of patients. Experimental colitis can be used to characterize immune pathways and address specific mechanistic questions, but using only animal models to predict responses of patients will identify incorrect therapeutic targets or could lead to dismissal of targets that might have potential for patients with IBD. Instead, to rapidly and appropriately identify therapeutic targets, animal models might be used as a screen for therapeutic efficacy, but other model systems, such as cell-based gene expression and functional assays that use samples from patients with IBD, should be incorporated into preclinical development.

Therapeutic Targets in Mucosal Immunity

The intestinal tract is the largest and most complex immune environment in the human body. Successful therapy for these tissues will require proper timing and location. In selecting location, many studies have shown that activated T cells mediate chronic intestinal inflammation. Cyclosporine A, an inhibitor of T-cell function, is effective in hospitalized patients with intravenous steroid-refractory severe ulcerative colitis (UC).[12] However, T cells are also required for defense against infectious pathogens, so inhibition of the entire population of T cells raises safety concerns.

Accordingly, a therapeutic strategy to inactivate activated T cells, without completely suppressing their activity, would be an innovative advance. T-cell activation requires T-cell receptor recognition of specific antigens in the context of a major histocompatibility complex molecule on antigen-presenting cells (APCs) and a costimulatory signal, derived from interaction of T-cell surface molecules CD28 and cytotoxic T lymphocyte associated antigen 4 (CTLA4) with B7 proteins on APCs. The fusion protein CTLA4-immunoglobulin (abatacept) binds the B7 costimulatory molecules on APC and prevents from delivering costimulatory signals to T cells. CTLA4-immunoglobulin has been approved by the FDA for treatment of rheumatoid arthritis (RA); it is highly effective in patients with moderate to severe RA who do not respond to TNF inhibitors.[13]

Because of success of TNF inhibition in patients with RA or IBD, some pharmaceutical companies have adopted the strategy that reagents that are effective against large market chronic inflammatory disorders such as RA, and psoriasis might also be successful for treatment of IBD. Therefore, following the demonstration of its efficacy in patients with RA, late-phase trials were initiated to test the effects of abatacept against UC and CD.

A case report published in 2006 indicated that CTLA4-Ig might have an unanticipated deleterious effect in IBD compared with RA.[14] A patient with a history of refractory RA who was included in a trial of CTLA4-Ig for this indication developed new-onset UC 15 months into treatment; 4 months after withdrawal of CTLA4-Ig, the patient was able to stop all UC therapies and remained asymptomatic. Unfortunately, this case report may have portended a disappointing outcome in a CD clinical study. A trial that tested the effects of CTLA4-Ig in 451 patients with moderate to severe CD who had increased levels of C-reactive protein (CRP) on enrollment had negative results. Interestingly, at early time points, there was even a trend that patients given CTLA4-Ig appeared to do worse than the placebo group. Effects of therapies in patients with RA therefore do not predict response of patients with IBD.

This was an important negative result from a mechanistic perspective. In certain strains of mice, the absence of B7 or CD28 results in de novo autoimmunity because of loss of T regulatory (Treg) cells.[15] Treg cells suppress immune responses through a variety of cell contact-dependent and -independent mechanisms. The transcription factor forkhead box P3 controls development and maintenance of Treg cells in mice and humans.[16] Mutations in forkhead box P3 or its regulatory regions cause Immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, characterized by large degrees of polyclonal T-cell

activation and inflammation. CTLA4-Ig disrupts costimulation of activated inflammatory T cells and also prevents the activation and/or function of Treg cells. The gastrointestinal (GI) tract is the largest reservoir of Treg cells in the human body, and Treg cells control tolerance to the enteric microbiota and dietary antigens.[17] Therefore, CTLA4-Ig might not have been successful against IBD because CTLA-4 signaling is required for Treg development and function in the GI tract.

Although IBDs are associated with T-cell activation, CD and UC have traditionally been distinguished by patterns of helper T-cell dysfunction. Lamina propria cells from patients with CD overproduce cytokines associated with a T helper (Th) 1 response, such as IL-12 and interferon-γ.[18] In contrast, cells from patients with UC overproduce cytokines associated with the Th2 response, such as IL-5 and IL-13.[19] Studies of mouse models of mucosal inflammation have provided further evidence that activities of Th1 and Th2 cells mediate pathogenesis of CD and UC, respectively. However, distinction of CD from UC based on over-production of Th1 and Th2 cytokines is an oversimplification of complicated immunology. Much of the inflammatory pathology originally believed to be mediated by Th1 cells and IL-12 was found to be mediated by a subset of T cells, Th17 cells, which produces the IL-17 family members IL-21 and IL-22 at sites of inflammation and require the IL-12 family member IL-23 as a growth factor.[20] Adding to the complexity, in various settings of mucosal inflammation, Th1, Th2, and Th17 cells have pro- or anti-inflammatory properties.[21] Therefore, although T cells are viable therapeutic targets for IBD, we have to be smarter about how (and when) to inhibit or induce the different subpopulations.

It has been a challenge to predict the efficacy of biologic agents in IBD trials; we have been surprised, if not blindsided, by unanticipated safety findings in the trials themselves and in postmarketing studies. Natalizumab was approved by the FDA because it is highly efficacious for patients with multiple sclerosis or CD; it binds to the integrin subunit α4, which is expressed on the surface of inflammatory cells.[22] By blocking α4, natalizumab prevents inflammatory cells from leaving blood vessels and entering sites of inflammation, such as the GI tract and the brain. Natalizumab use has been markedly curtailed because of an unanticipated complication, progressive multifocal encephalopathy (PML)–a serious, often fatal, infection of the brain caused by reactivation of the JC polyoma virus. PML has been reported in approximately 0.1% of patients treated with natalizumab for more than 1 year.[23] Ironically, this significant adverse event has informed subsequent approaches and might lead to more specific, safer therapeutics. Approaches that specifically prevent inflammatory cells from entering the GI tract but do not disrupt immune surveillance in the brain (the major predisposing factor for PML) have shown promise in early-stage trials. α4 Can heterodimerize with β1; the resulting integrin $\alpha4\beta1$ binds the vascular endothelial ligand-1, which is expressed at sites of inflammation, including the brain. However, the integrin $\alpha4\beta7$ is GI specific and binds the monoclonal antibody vedolizumab, which is in phase 3 trials for UC and CD.[24,25] An antibody against $\beta7$ is also in early-phase studies of CD, and an antibody to the GI-specific vascular endothelial ligand of $\alpha4\beta7$, mucosal vascular adressin cell adhesion molecule-1, has shown promise in early-stage trials.

Targeting Therapies to Sites of Inflammation

Development of biologic agents that target specific defects associated with IBD, as opposed to the sledgehammer of immunosuppression, is a goal of future therapy. An important innovation would be to find a way to deliver potent therapeutics directly to the intestinal tract. Targeting therapies to sites of GI inflammation is a challenge because there are no vehicles that can carry sufficient amounts of the article and release it at the proper GI location with minimal degradation by digestive enzymes or systemic absorption. Phase 3 trials of IL-10 for IBD were stopped because of lack of efficacy. The therapeutic efficacy of recombinant IL-10, delivered by injection, might be limited, in part, by its poor bioavailability to GI tissue and adverse effects at higher concentrations. The efficacy of IL-10 might be improved by localized delivery of IL-10 to the GI tract, which would minimize systemic exposure and toxicity. To test this hypothesis, researchers engineered the enteric bacteria *Lactococcus lactis* to secrete IL-10; when these bacteria were administered to *IL-10*$^{-/-}$ mice, they transiently colonized the GI tract where they produced IL-10 and prevented inflammation.[26] This finding led to a phase 1 trial for CD in which the IL-10-expressing bacteria were tolerated, but their efficacy has not been determined.[27]

Gene and nucleotide-based strategies might be used to overcome the technical challenge of delivering effective therapies to the intestine. An advantage of gene therapy is that genes can be delivered to local sites, produce and concentrate a therapeutic protein in intestinal tissue, and release negligible amounts into the circulation. Rectal administration of a nonreplicating adenoviral vector that expressed mouse IL-10 reduced symptoms and histologic features of inflammation in *IL-10*$^{-/-}$ mice.[28] However, there are concerns about the safety of viral vectors in humans, including endogenous virus recombination that allows replication of competent viruses and host immunogenic reactions to viral particles, which can lead to ineffective, repeated dosing. Therefore, nonviral methods of gene transfer to the intestine might be more feasible approaches to gene therapy for IBD. For example, polymeric nanoparticles are specifically taken up by inflamed tissue, so numerous small molecules might be delivered directly to and concentrated in target tissue. Recently, nanoparticle delivery of the anti-inflammatory tripeptide lys-pro-val (KPV) to the colon reduced dextran sulfate sodium-induced colitis in mice.[29]

Antisense oligonucleotides and short interfering RNAs (siRNAs) might be delivered to prevent expression of proinflammatory genes associated with IBD. Enemas that deliver alicaforsen, a 20-base pair phosphorothiolate antisense oligodeoxynucleotide that binds to a 3′ untranslated region of human *intercellular adhesion molecule-1* messenger RNA (an adhesion molecule that mediates the inflammatory response), are being tested in a phase 2, placebo-controlled study of patients with mild to moderate, left-sided UC.[30] Small, double-stranded RNA sequences (siRNA or short hairpin RNA) might be developed as nucleic acid-based therapies. In the cytoplasm, siRNAs initiate a process that cleaves a complementary messenger RNA to prevent its processing and translation. Several studies have shown that delivery of siRNA-containing nanoparticles directly to the GI tract of mice reduce colitis. Local delivery of nanoparticles that contain an siRNA against *TNF* reduced dextran sulfate sodium-induced colitis in mice.[31]

Cell-Based Therapeutics

Autologous transplantation of hematopoietic stem cells was the first cell-based attempt to treat inflammatory disease; it has been tested in patients with active refractory RA, juvenile idiopathic arthritis, multiple sclerosis, and IBD.[32] Although it led to prolonged responses in some patients, immunosuppression significantly increased risk of infection and even mortality. This approach is effective because it involves ablation and replacement of the host immune system, eliminating inflammatory T-cell responses, generating naïve T cells, and, in a sense, rebooting the immune system. However, the genetic features that lead to chronic immune activation are not eliminated, so, if patients encounter environmental activators of inflammation, disease can recur.

Interestingly, Treg cells are believed to be an important component of the immune system that develops from the transplanted cells. An intriguing approach to increase numbers of Treg cells without myeloblation might be to isolate Treg cells from patients, expand them in culture, and then infuse them back into patients. Ex vivo expansion and then infusion of Treg cells have prevented or reversed inflammatory diseases in several preclinical models of IBD.[33] Other tolerogenic types of immune cells, such as dendritic cells, can also be expanded ex vivo for manipulation and potential therapy.[34]

Stem cell-based therapies hold promise but raise controversy. Important properties of stem cells include self-renewal (they undergo repeated cell division cycles but maintain an undifferentiated state) and potency (they differentiate into specialized types of cells). Embryonic stem cells are obtained from blastocyts, whereas adult stem cells are found in all adult tissues. Adult stem cell-based therapies have been tested in patients; those that have been included in trials for IBD or other inflammatory diseases are multipotent–they can differentiate into other cell types. Bone marrow contains hematopoietic, endothelial, and mesenchymal stem cells (MSCs). Hematopoietic stem cells differentiate into all types of blood cells and are used in autologous and allogeneic bone marrow transplants. MSCs are undifferentiated, multipotent cells that also reside in the bone marrow. In preclinical models, MSCs differentiate into cells that can reduce the effects of inflammatory disease. They can down-regulate immune responses through intrinsic properties, such as production of IL-10. They can also promote epithelial cell repair in the GI tract and promote tissue repair by stimulating formation of new blood vessels.[35] Studies of MSCs for treatment of CD are underway.

Although cell-based therapies are exciting mechanistically, they present clinical challenges. Cells can differentiate into pathogenic subsets in vivo. The pharmacokinetic properties of small molecule reagents can be easily assessed in people, but cell-based therapies have no easily quantifiable parameters for monitoring, such as how many cells need to be given, how frequently, where the cells go, and what they differentiate into. Moreover, other than clinical responses, there are no clear pharmacodynamic parameters that could be used as end points in trials. Despite the interest, cell-based therapies should be regarded with cautious optimism because technical challenges remain.

Personalized Medicine

The number of therapeutics in development for IBD has increased dramatically over the last 2 decades because of rapid gains in our understanding of mechanisms of inflammation. There are now more than 50 products directed against nearly as many targets. These products include monoclonal antibodies, small molecules, siRNA/short hairpin RNA, peptides, vaccines, and cell-based therapies (Melmed and Targan[36] and references therein). Although the number of potential therapeutics in the pipeline is increasing, drug development can be hindered by conventional study designs, regulatory issues, and, importantly, by the small number of patients willing to participate in trials for IBDs. Personalizing medicine for patients with IBD might require significant changes to the drug development and testing process.

A fundamental concern is the requirement for large study populations in clinical trials. As the list of therapeutic reagents grows, there will not be sufficient patients to participate in each trial, if each study requires 1500 patients, which was the case for development of natalizumab. Even though 500 patients participated in a phase 3 trial and 1000 patients were enrolled in a phase 2b dose-finding study, the absolute differences in response and remission between placebo and the test article were 16% and 10%, respectively,[37] and there was much initial doubt whether natalizumab would be approved by the FDA and brought to the market.

Consequently, future clinical trial design will be better informed through the use of biomarkers and genotypic information,[38] equivalent to the 21st century concept of personalized medicine. Study populations would be preselected based on genetic variants and biologic markers to stratify groups of patients most likely (and least likely) to respond to a therapeutic intervention. Such an approach takes into consideration the diversity of pathobiology observed among patients with UC or CD.

Utilizing this approach to clinical trials involves the use of recent findings about the immunopathogenesis of IBD.[39] Pathogenesis of IBD involves a combination of genetic factors and dysregulated innate and adaptive immune responses to environmental factors, which can include the commensal luminal microbiota. There are many reagents, mostly monoclonal antibodies, directed at different factors in these processes, including cytokines, surface receptors (including those that regulate cell localization), and signaling molecules.

The challenge is to determine which molecules and populations of cells are the best therapeutic targets for different subgroups of patients, based on their genetic and biologic factors. For example, reagents designed to disrupt IL-23 and IL-12 signaling were more effective for patients with some inflammatory diseases than others, despite results from several animal models of inflammation.[40] The inflammatory cytokines IL-12 and IL-23 comprise a common p40 subunit, covalently linked to the p35 and p19 chains, respectively. IL-12 and IL-23 are involved in inducing and maintaining distinct, inflammatory T-cell responses, via Th1 and Th17 cells, respectively. Antibodies that specifically recognize the p40 subunit were therefore predicted to be effective against inflammatory disorders. Therapies that were effective for psoriasis have been predicted to also be effective for patients with IBD; genetic factors that increase risk for psoriasis (variants that affect IL-12

or IL-23 signaling) also increase risk for CD and UC. Drugs that block IL-12 and IL-23 activity were effective in trials of patients with psoriasis; 60% of patients with psoriasis had greater than 90% improvement in disease activity, compared with none of the patients given placebo.[41–43] Ustekinumab, a human immunoglobulin G1 against p40, has been approved for treatment of moderate to severe psoriasis in the United States, Canada, and Europe. In contrast, its results in patients with CD were disappointing because the study did not achieve the primary end point. Post-trial analysis, however, indicated that patients who had been previously treated with anti-TNF agents might respond better than those who had not.[44] A trial of a different antibody against p40 in patients with CD that did not include subgroup analysis found no significant difference in the primary end point (remission) between patients given the test article or placebo.[45]

The question for scientists is why would an agent that blocks pathways required for mucosal inflammation be effective in patients with psoriasis but not CD? Explanations include that higher doses might be required to achieve target saturation in the intestine, that clinical and biologic end points for IBDs need to be better defined, or that the study population included too many patients with severe disease. Pathogenesis of psoriasis and IBDs might involve different pathways of inflammation; p40 might be required for progression of the inflammatory response that leads to psoriasis but not IBDs. Recent technologic advances in genetic analysis have shown that IL-23 and possibly IL-12 are the primary mediators of inflammation in patients with psoriasis but that heterogenous factors are associated with the inflammatory response in patients with CD. It is possible, then, that a subpopulation of patients with CD mediated predominantly by IL-12, IL-23, and IL-17 would respond to therapeutic antibodies against p40.

Many genetic variants are shared between patients with UC and CD, which calls into question whether there is any utility in dividing inflammatory bowel diseases into these taxonomic classifications. By 2008, genome-wide association studies (GWAS) had associated more than 30 different genes and loci with IBD,[46] and now more than 70 have been associated with CD and 50 with UC–many of which overlap.[47]

GWAS have been refined to include information on specific traits, such as disease behavior or biomarkers, in the analysis. For example, a GWAS found that specific variants in the gene encoding the IL-23 receptor and variations in genes that encode other factors in the signaling pathway increase susceptibility to CD.[48] Many studies are needed to understand better how specific genetic variants affect disease expression. The IL-23 pathway seems to be genetically more important in a broader group of patients with psoriasis. In contrast, in CD, the subgroup of patients in which IL-23 is most important appears to be smaller. Fewer genetic variants have been associated with psoriasis, with much stronger associations and less variation in the IL-23 pathway. GWAS cannot, of yet, associate specific genetic factors with response to a specific therapeutic.

Many studies are needed to determine how the products of the genes identified in GWAS affect risk for IBDs–to determine how they modify immune mechanisms and contribute to pathogenesis. The number and types of genetic variations and their effects on processes could determine how IBDs develop and progress, how mild or severe a disease course is, and

whether they are likely to respond to a particular therapeutic. Analyses of serum immune responses, alone or in combination with information on clinical features, might allow stratification of populations to improve efficiency of clinical trials and reduce the number of patients who need to be enrolled in each trial. Specific genetic variants might be associated with specific disease phenotypes, severity of disease, or response to a reagent that targets a particular pathway. One genetic variant could cause a certain level of disease severity, whereas other variants might have combined effects, leading to the same degree of severity. The number of possible combinations of clinical findings, biomarkers, and genetic variants associated with IBDs might account for the lack of efficacy of anti-p40, and potentially other therapeutics, in the broad population of patients with CD.

Mutations in genes that encode subunits of the IL-10 receptor have been associated with severity of CD.[11] Patients in this study also developed severe skin manifestations, similar to that observed in *Hidradenitis suppurativa*, a skin condition associated with CD. The patients were resistant to all therapeutics but at least 1 responded to stem cell transplantation because the transplanted cells carried *IL-10* without the disease-causing mutation. What distinguishes these patients is that they had none of the more than 50 other CD-associated genetic variants known at the time. In contrast, patients with numerous UC-associated genetic variants develop a more severe disease phenotype that is resistant to treatment with cyclosporine, anti-TNF, immunomodulators, and corticosteroids.[49] Patients with combinations of UC-associated variants require surgery sooner than patients with fewer variants, for whom surgery is rare.[49]

Over the past 20 years, analyses of serum samples from patients have associated immune responses to certain commensal bacterial antigens with CD and UC.[50–53] Protoplasmic-staining antineutrophil cytoplasmic antibodies appear to cross-react with bacterial structures in that immunofluorescence can be absorbed by prior incubation with cecal bacterial extracts.[54] Antibody responses against microbes have been associated with certain clinical phenotypes and treatment responses. Furthermore, it appears that the number and magnitude of these immune responses are associated with progression of UC and might predict complications, such as chronic pouchitis following ileal pouch anal anastomosis.[50,55] Most importantly, the antibodies detected reflect the magnitude and types of mucosal dysfunction, which could result from different genetic variants. Specific antibodies in serum, individually or a combination, have been associated with different features of severe CD, such as fibrostenosis, internal penetrating disease, or need for small bowel surgery. Because each type of immune response is independently associated with a different feature of severe CD,[50,51] they might be related to different and even nonoverlapping pathways of immune regulation. In prospective studies of young children, those with a greater number of antibacterial antibodies required surgery sooner than those with fewer or no antibodies.[53] Mice with disrupted innate immune responses do not develop spontaneous colitis, but, when antibody responses to certain commensal antigens are measured, there is a marked increase in peripheral antibodies to these antigens suggesting an enhanced adaptive immune response.[56]

Combined analyses of clinical phenotypes, serotypes, genotypes, and gene expression profiles of peripheral and mucosal cells might be used to predict responses to particular

therapeutics. Studies in a pediatric population indicated that combinations of genes, serotypes, and clinical phenotypes can predict which patients will not respond to anti-TNF.[57] Analyses of genetic variations and serologic responses can identify specific features of immune pathways of groups of patients; additional biomarkers of the state of inflammation at a specific time point might increase sensitivity and specificity of these analyses. Using microarray analysis, Arijs et al showed that mucosal expression of 5 different immune response genes were able to identify patients most likely to respond to anti-TNF.[58]

It is important to use data from basic science studies in the design of clinical trials, using the data from early phases of drug development to select the end points and foci of trials. Studies of the TNF superfamily member 15 (TNFSF15) demonstrated how serologic and genomic data can be used in development of clinical trials. A GWAS associated small nucleotide polymorphisms in *TNFSF15* with CD and UC, making this the first gene to be associated with both IBDs,[59] in all ethnic groups analyzed.[60] Patients with these small nucleotide polymorphisms also had overexpression of the product TNF-like ligand-1A (TL1A) in patients with CD and some patients with UC, but levels varied among individuals with equivalent levels of inflammation.[60] TL1A is a cytokine that regulates mucosal inflammation and the development and amplitude of Th1-, Th17-, and Th2-cell responses.[61] Genetic studies that performed haplotype analyses of *TNFSF15* in different ethnic groups associated specific haplotypes with more severe forms of CD and expression levels of membrane and soluble TL1A in monocytes.[60,62]

Another association between *TNFSF15* and disease severity was made in a GWAS of patients with UC who did not respond to therapy. In this study, *TNFSF15* almost reached genome-wide significance, indicating that TL1A might be developed as a therapeutic target for patients who do not respond to anti-TNF or other immunomodulators.[57] In models of chronic inflammation, reagents that block TL1A prevented and reduced chronic inflammation by inhibiting Th1- and Th17-cell responses.[61] If a therapeutic agent that inhibits TL1A is developed, analyses of variants in *TNFSF15* might be used to identify patients most likely to respond. Trials for an anti-TL1A therapeutic might have a higher likelihood of well-defined efficacy if study patients are selected based on variants in *TNFSF15* or overexpression of TL1A.

Conclusion

Clinical trial strategies should be modified to account for differences in pathogenesis of IBDs. Genetic and other types of biology studies will continue to identify potential therapeutic targets and pathways. Data from genomic, gene expression profiling, and serologic analyses, along with carefully selected clinical information, should be used to design phase 2 trials and select patient populations. This approach will help identify the most appropriate subjects for inclusion in phase 3 trials prior to enrollment and/or improve our ability to define responders and nonresponders as an end point for phase 3 trials using an unselected population of patients. Definitions of new end points for clinical trials are likely to become increasingly controversial.

Trial design and, in particular, end point selection have not evolved at the same rate as technology. The classical end points of clinical remission and steroid independence were replaced, to some degree, by specified changes in disease indices (eg, greater than or equal to 100 point change in CD activity index). Recently, the end points of endoscopic confirmation of mucosal healing and/or reduced inflammation, defined by levels of CRP, have gained popularity because they are rigorous determinants of response. These end points, however, could limit the potential for defined efficacy for therapeutics aimed at specific pathobiologic targets. In designing trials of reagents that target a specific factor or pathway required for inflammation, subjects should be selected (for the active treatment and placebo groups) who have known alterations in this factor or pathway: they are more likely to respond, compared with the overall population of patients with IBDs. Mucosal healing and changes in levels of CRP are suitable end points for these types of studies.

Reagents that have been abandoned because they were found to have minimal or insufficient efficacy in trials might be re-evaluated using populations most likely to respond. In addition, further analysis of results from previous trials, such as those from trials of ustekinumab, might provide insight into the genetic and immunologic features of the study population, indicators of mucosal and peripheral inflammation, and information about when to initiate therapy. The potential of personalized medicine can be tested by redesign of the clinical trial process (Table 1).

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Abbreviations used in this paper

APC	antigen-presenting cells
CRP	C-reactive protein
CTLA4	cytotoxic T lymphocyte associated antigen 4
FDA	Food and Drug Administration
GI	gastrointestinal
GWAS	genome-wide association study
IL	interleukin
MSC	mesenchymal stem cells
PMA	progressive multifocal encephalopathy
RA	rheumatoid arthritis
siRNA	short interfering RNA
Th	T helper
TL1A	TNF-like ligand-1A
TNF	tumor necrosis factor
TNFSF15	TNF superfamily member 15
Treg	T regulatory cells

Table 1

Status of Selected Clinical Development Programs of Novel Therapeutic Approaches in IBD

	Cytokines/growth factors	Adhesion molecules/chemokines	T cells	Others
Approved (in US)	• Anti-TNF mAb (infliximab, adalimumab, certolizumab pegol)	• Anti-α4 integrin mAb (natalizumab)		
In clinical development	• Anti-IL-12/23 mAb • Anti-IL-23 mAb • Anti-IL-17 • IL-6 inhibitors • Other TNF inhibitors	• Anti-$\alpha4\beta7$ mAb • Anti-$\beta7$ mAb • Anti-MADCAM mAb • CCR9 antagonist		• Stem cel • Autologo marrow t • Janus kin inhibitor • Helminth • Probiotic • Antibioti
No longer in clinical development (failed)	• IL-10 • IL-11 • Soluble TNF receptors • IL-1 receptor antagonist • Anti-interferon-γ mAb • GM-CSF • Keratinocyte growth factor • Oral IL-12 inhibitor		• CTLA4 Ig • Anti-CD3 mAb • Anti-CD25 mAb	• MAP kin

mAb, monoclonal antibody.

NOTE. This list is not comprehensive but highlights several of the targets and approaches discussed in this article. As for some targets (eg, new TNF inhibitors, IL-6 antagonists, Janus kinase inhibitors), several compounds are currently in development, and approaches are categorized by target rather than specific compound nomenclature (with the exception of FDA-approved compounds). Compounds listed as “no longer in clinical development” were categorized based on negative phase 2 or 3 clinical trial results that have been reported in the public domain and the absence of active enrolling studies listed on www.clinicaltrials.gov. This categorization also may not account for clinical trials being conducted exclusively outside of the United States. Moreover, as discussed in the article, there can be many reasons why compounds are no longer in clinical development, and inclusion on this list does not necessarily imply that these targets are irrelevant for future development in IBD. Finally, because this is a rapidly moving field, this Table will be subject to constant revision as clinical trial results are released. The reader is referred to www.clinicaltrials.gov for further information on the status of ongoing clinical trial programs.