Challenges in IBD 2019

by

Crohn’s & Colitis Foundation

and the Challenges in IBD working group*

Abraham, Clara; Afzelius, Lovisa; Arora, Manish; Bemelman, Willem; Bousvaros, Athos; Boyle, Brendan; Braun, Jonathan; Brodmerkel, Carrie; Burns, Richeal; Cannon, Lisa; Chang, Eugene; Cho, Judy; Chuang, Emil; Colombel, Jean-Frederic; Cortes, Constanza; Cowden Dahl, Karen; Croitoru Ken, Karen; Cross, Ray; Curran, Mark; D’Hoore, Andre; De Zoete, Marcel; Delaney, Conor; Denson, Lee; Dobes, Angela; Donowitz, Mark; Dubinsky, Marla; Duerr, Richard; Dhyani, Manish; Elson, Charles; Faith, Jeremiah; Fichera, Alessandro; Fleshman, James; Garrett, Wendy; Gregerson, Peter; Guillory, Ashley; Ha, Christina; Heller, Caren; Ho, Shuk-Mei; Hodin, Richard; Honig, Gerard; Hurtado-Lorenzo, Andres; Hyams, Jeffrey; Jerret, Michael; Joshi, Nitin; Judson, Richard; Karp, Jeffrey; Kellis, Manolis; Kim, SandraSandra; Koltun, Walter; Korzenik, Joshua; Kischer, Jeff; Kugathasan, Subra; Lasch, Karen; Lee, Jessica; Grant, Melanie; Lewis, James; Lineberry, Neil; Long, Millie; Lu, Tim; Mahadevan, Uma; Mayer, Emeran; Mazmanian, Sarkis; McGovern, Dermot; Michelassi, Fabrizio; Miller, Theresa; Mostoslavsky, Gustavo; Nduaka, Chudy; Newberry, Rodney; Niessen, Heiko; Park, KT; Peer, Dan; Pekow, Joel; Pereira, Maria; Pizarro, Theresa; Plevy, Scott; Polk, D. Brent; Poritz, Lisa; Rafferty, Janice; Rappaport, Stephen; Relman, David; Robinson, Anne; Rosen, Michael; Ruben, David; Salzman, Nita; Sartor, Balfour; Scott, Frank; Shtraizent, Nataly; Siegel, Corey; Snapper, Scott; Stappenbeck, Thaddeus; Sylvester, Francisco; Towne, Jennifer; Traverso, Giovanni; Virgin, Herbert; Weaver, Aandra; Wu, Gary; Yajnik, Vijay.

*Displayed in alphabetical order
Table of Contents

PREFACE

INTRODUCTION: THE CHALLENGES IN IBD PROCESS

CHAPTER I: PRECLINICAL HUMAN IBD MECHANISMS
   A. Triggers of Immune Response
   B. Intestinal Epithelial Homeostasis and Wound repair
   C. Developmental and Age-Related Pathophysiology
   D. Biology of Complications
   E. Biologic Determinants of Disease Location
   F. New Therapies and Response to Treatment

CHAPTER II: ENVIROMENTAL TRIGGERS
   A. Epidemiology of Exposures and Disease Evolution: Concepts in Study Design
   B. Signatures of Biological Response to Exposures
   C. Mechanisms of How Environmental Exposure Drive IBD

CHAPTER III: NOVEL TECHNOLOGIES
   A. Non-invasive Detection and Evaluation of Active Inflammation in IBD: Imaging and Sensing Technologies
   B. Technologies to Enable Mucosal Targeted Drug Delivery Systems that Enhance Efficacy and Decrease Side Effects
   C. Surgical and Other Technologies to Prevent Post-operative Septic Complications, Treat Fistulizing Disease and Prevent Anastomotic Failure

CHAPTER IV: PRECISION MEDICINE
   A. Understanding the Natural History of IBD: Disease Susceptibility, Activity, and Behavior
   B. Predicting Drug Response
   C. Optimizing Current and Developing New Molecular Technologies to Enable Precision Medicine

CHAPTER V: PRAGMATIC CLINICAL RESEARCH
   A. Better Understanding the Incidence and Prevalence of IBD in the United States
   B. More Accurate Medication Selection for Patients with Inflammatory Bowel Disease to Increase Treatment Effectiveness
   C. Better Defining How Clinicians are Utilizing Therapeutic Drug Monitoring in IBD
   D. Understanding of Healthcare Resources Gaps and Utilization: Pain Management, Psychiatric Services, and Alternative Medicines
INTRODUCTION: THE CHALLENGES IN IBD PROCESS

Millions of patients in the U.S. and worldwide suffer from Crohn’s disease and ulcerative colitis, with a continued dramatic increase in incidence largely associated with industrialization. There is an urgent need to optimize the use of current therapies and develop new ones with the ultimate goal of preventing and/or finding a cure for these diseases. Ever mindful of its primary mission, and with renewed vigor, the Crohn’s & Colitis Foundation is focused on providing tangible deliverables to its most important stakeholders: Patients and their families. Motivated by the urgency by which results must be delivered, the Foundation has undertaken a fresh approach to identifying scientific priorities to guide its research portfolio, placing a greater emphasis on innovation, multi-disciplinary teamwork, and patient-centricity.

Innovation is the cornerstone of any successful research enterprise and investigator-initiated research is the foundation upon which most advances in biomedical research are facilitated. Nevertheless, a more targeted engineering approach, viewing a biological question as a problem to be solved by assembling the optimal minds together with the utilization of advanced technology, is becoming an increasingly effective approach to accomplish significant scientific advances. In addition, teamwork, building upon the notion of multidisciplinary research, is a concept that is often critical in leveraging synergistic interactions in an increasingly technology intensive scientific environment. Although often considered a critical component within the domain of basic researchers, essential members of a team focused on deliverables in biomedical research require partnerships between basic scientists, clinical researchers, and clinicians. It is important to bridge the gap between clinicians and basic scientists so that clinicians understand the importance of basic research and basic scientists value the insights and contributions of physicians who care for patients. It is also important to emphasize the concept of “bedside to bench” research, as an organizing idea driving the biological question to be addressed, since it can increase the likelihood that preclinical research findings will have applicability to the human disease and enable acceleration of the translation of these findings to patients.

This focus on “bedside to bench” and translational research can support a similar synergistic partnership between academia and industry. To be successful, industry must develop products that meet the rigorous standards for efficacy and safety established by regulatory agencies such as the FDA. A forward-thinking program in academia can be a powerful ally as a partner to industry in achieving this goal. In return, industry offers enormous tangible deliverables to academia that can help basic and clinical scientists “reduce-to-practice” innovations discovered at the wet bench or in the clinics. These types of partnerships will accelerate deliverables into the clinical domain to develop innovative approaches to diagnose and treat disease.

With these notions in mind, the Foundation charged a group of key opinion leaders of scientists, clinicians, and members from industry, along with patients and caregivers, to identify the most important biological questions, or gaps in knowledge that could be addressed in the next three to five years to
accelerate the timeline needed to achieve cures and new therapies for patients with Crohn’s disease and ulcerative colitis.

Approximately 100 experts and stakeholders, divided into workgroups, addressed five focus areas related to IBD: 1) Preclinical human IBD mechanisms; 2) Environmental triggers; 3) Novel technologies; 4) Precision medicine; and 5) Pragmatic clinical research. In each of these areas, the workgroup addressed the following general questions:

- What are the hurdles preventing the advance towards better treatments and diagnostics?
- What are the priority topics to be investigated?
- What tools are needed to accelerate these areas?

After summarizing the findings of the various workgroups, a draft was made available for public comment culminating in this “Challenges in IBD” document. We hope that the ideas in the following pages will serve as a stimulus to focus research efforts on addressing the most important gaps in knowledge that can begin to be addressed in the next three to five years so that practical solutions that will touch the lives of patients suffering from IBD can be developed more rapidly.
CHAPTER I: PRECLINICAL HUMAN IBD MECHANISMS

The focus of preclinical human mechanisms research is the identification and study of questions and processes that are most highly relevant to patients with IBD. This requires the connection of the basic science approaches to human IBD. Notable and impactful advances have been made over the last several years, however several knowledge gaps remain. The highest priority gaps that have been identified are in areas of (1) triggers of immune response, (2) intestinal epithelial homeostasis and wound repair, (3) developmental and age-related pathophysiology, (4) biology of complications, (5) biological determinants of disease location, and (6) new therapies and response to treatment.

A. Triggers of Immune Response

It is widely accepted that IBD is the result of dysregulated gut mucosal immune responses to environmental factors in genetically-predisposed individuals. For several years, immune dysregulation was primarily attributed to an imbalance between pathogenic and regulatory T cells, and emphasis was placed on the adaptive arm of the gut mucosal immune system in the pathogenesis of IBD. However, although aberrant adaptive immunity is still thought to play an essential role, more recent studies have focused on upstream events, based on reports highlighting the importance of functional and genetic associations between dysregulated intestinal innate immune responses and IBD [1]. Environmental triggers primarily affecting early innate immune responses, including the gut microbiome, diet, and enteric infections, among others, are currently primary areas of research in identifying etiologic and modifying factors in the pathogenesis of IBD. Indeed, the rapidly increasing incidence of IBD associated with industrialization emphasizes the importance in environmental factors in the pathogenesis of IBD [2]. IBD is characterized by alterations in the composition of the intestinal microbiome [3]. In addition, IBD patients with active disease have been reported to have a different microbiome composition compared to patients in remission [4]. While these general concepts have been firmly established, there are major gaps that exist in dissecting the specific impact of the gut microbiome in IBD. This includes the need to identify specific microbial metabolites and bacterial strains, as well as global patterns thereof, and their functional roles in regulating gut mucosal immune responses. It is also important to recognize that microbes may not possess the same triggering effects on immune responses throughout the entire gastrointestinal tract, and that their effects may be dependent on anatomic location within the gut mucosa. Also, while it is easy to conceptualize how the host's immune system can innately respond to various microbes and their microbial products, not much is known about how these commensal microbes react to components of the gut mucosal immune system and how this process may differ in patients with IBD. Other possible environmental microbial triggers include enteric infections and the nonbacterial microbiota (i.e. fungi and viruses) and their roles in triggering immune responses leading to IBD, which is an active area of investigation. There is also a gap in knowledge about the interrelationship between members of the microbiome and dietary components, and the subsequent...
host response to this interaction. Finally, uncovering the precise relationship between diet and the microbiome and how this relationship reflects on immune response is an active and highly warranted area of investigation.

B. Intestinal epithelial homeostasis and wound repair

Understanding the essential role of the intestinal epithelium in IBD is paramount, particularly since mucosal healing has become a major endpoint in clinical management of patients. Research in this area has focused on epithelial intrinsic defects, extrinsic effects on epithelial function, and alterations of the intestinal epithelium during repair.

Recent translational studies have revealed a rich and still expanding set of genetic polymorphisms/mutations in IBD subjects. These discoveries have launched investigations into epithelial-intrinsic defects that may be instrumental in understanding the mechanisms of IBD pathophysiology. In addition, the nature of the gene mutations that lead to susceptibility to IBD also depend on environmental factors that are challenging to uncover in human subjects. A plethora of new techniques, including systems biology and mechanistic approaches, can be integrated to study the link between human genetic polymorphisms/mutations and specific pathways relevant to IBD. The current approach to account for genetic complexity and to leverage advances in IBD genetics is to select genes and proteins for detailed study based on informative mutations associated with susceptibility or resistance to IBD, and to study these genes and proteins in relationship to each other [5, 6].

An emerging area of challenge is to define precisely the effects of non-epithelial factors from other mucosal cells that impact cell function as above. The current approach is the co-culture of microbes, microbial product and immune cells to model interactions that occur in vivo [7, 8]. There are many platforms that are in development from traditional organoids (primary in IPS derived), spheroids that are enriched in specific cell types [9], gut-on-a-chip systems with controlled shear and oxygen parameters [10], and Transwell cultures [11]. Each of these platforms will provide unique opportunities to query epithelial interaction. Key approaches to studying the effect of environmental factors in the context of gene mutations that lead to susceptibility of IBD in human patients have included integration of insights from patient data; knockout (KO) and knock-in (KI) mouse studies; mechanistic studies; and network analysis to define pathways involved in the phenotypes of human cells carrying risk mutations. In this way, one can link standard mechanistic studies to studies in patient cells to rapidly move to clinical relevance. Specific to the epithelium, there are several cellular targets and processes that remain of high interest to IBD pathophysiology and therefore warrant further careful investigation. The major epithelial cell type is an absorptive barrier cell (enterocyte in the small intestine and colonocyte in the colon). Effects on metabolism, transport, barrier function and differentiation are of high interest and are still poorly understood in the context of IBD. For secretory lineages, there is a much deeper appreciation of the potential role of mucus in animal models, though study of this has been challenging to test in human subjects. There is also a new
appreciation for various functional sub-lineages of goblet cells that detect microbial products and direct secretion of cargo [12]. Such functional sub-lineages occur for other secretory lineages, and this will be a rich source of future research. For example, this approach should better define specific pathways in IBD that affect Paneth cell function [13], in particular production and release of antimicrobial proteins, enteroendocrine lineages [14] and the newly invigorated tuft cells that can sense and react to specific types of infections in the intestine [15]. The suggestion from initial single cell RNA sequencing of the intestinal epithelium is that there are multiple sub-lineages present for certain morphologically defined lineages [16]. Additional functional verification of these sub-lineages in this context will be valuable. The challenge is now to devise methods to test the sub-lineages that are present.

Intestinal wound repair is a tightly orchestrated event that occurs in sequential phases that are highly involved the epithelium. Using experimental systems for studying injury of the intestinal mucosa has allowed investigators to demonstrate that wound repair occurs in a defined time and place, and to focus on epithelial changes and timing that occurs during repair of injuries [17-22]. The challenge is to determine whether there are alternative factors that can drive this process, particularly during inflammation and chronic damage. To better understand the special and temporal requirements for mucosal healing, currently existing experimental models of intestinal injury and in vitro modeling could be useful to screen for new factors [23, 24]. What remains unclear is the role of the intestinal microbiome given the alterations in the microbiome the wound site [25] and what microbiome related mechanisms can prevent penetrating injuries.

C. Developmental and age-related pathophysiology

In recent years, our understanding of the early development of the gut microbiome and factors affecting IBD onset and progression early in life has advanced significantly. Investigators discovered rare mutations in over 50 genes interfering with both epithelial and immune functions that lead to high penetrant forms of very early onset IBD (VEO-IBD) [26]. For a handful of these mutations, advances in our understanding of their pathogenic mechanisms have led to development of therapeutics with allogeneic hematopoietic stem cell transplant (e.g. mutations in the IL-10 receptor and XIAP) or successful treatment with emerging drugs targeting the affected pathway (e.g. abatacept for defects in CTLA4 or LRBA). Advances in DNA sequencing technology led to an explosion in our understanding of the dynamic nature of the early life gut microbiome and the strong effects of Caesarean section, early life nutrition, and antibiotics on species abundance and diversity [27]. The large RISK inception cohort yielded a detailed characterization of the profound dysbiosis present in treatment-naïve children with Crohn’s disease, and investigators have begun to characterize microbiome dynamics as they relate to inflammation, nutrition, antibiotics, and anti-TNF treatment in pediatric IBD [28, 29]. The RISK study increased our understanding of the gut microbial alterations and tissue transcriptional programs in treatment-naïve pediatric Crohn’s disease patients that portend risk for structuring and penetrating complications [30].

To build on these achievements and meaningfully advance our understanding of the developmental and age-related pathophysiology of IBD, the scientific community must address several critical knowledge
gaps. With regard to VEO-IBD, the last decade focused on single gene defect identification, but a specific causative gene mutation is only identified in 20 percent of patients [26]. We must broaden our understanding of the mechanisms through which high penetrance gene mutations and gene interactions cause VEO-IBD so that we can develop new treatments and repurpose existing therapies. Additionally, the incidence of IBD is rising disproportionately in these youngest children [31]. A small peak of IBD diagnoses late in life has been long established, yet we do not understand the environmental or microbial triggers of IBD at these developmental extremes. Additionally, since the mucosal immune system is ultimately the effector of the chronic inflammation of IBD, it will be critical to understand how the key components of the immune system change with age to affect risk and presentation of IBD across the age spectrum. Lastly, environmental influences and host genetics intersect to influence disease in the burgeoning field of epigenetics. In the coming years, we must scale our understanding of how the cell-type-specific accumulation of epigenetic changes across the life span influences the development of IBD at specific developmental windows.

D. Biology of complications

More than 50% of patient with Crohn’s disease (CD) develop complications over their lifetime, defined as stricturing (luminal narrowing, wall thickening and prestenotic dilation) or penetrating (an abnormal passage between the intestine and the body surface or other organs) disease [32, 33]. Strictures disease is a serious problem also for ulcerative colitis (UC) with approximately 8% incidence over the lifetime of disease [34]. The importance of studying fibrostenosis is emphasized by the facts that its pathophysiology is complex, dynamic, multifactorial, and involves multiple cell types. Current anti-inflammatory therapies can neither prevent nor treat fibrosis; no specific anti-fibrotic therapy is available; no validated predictors for intestinal fibrostenosis have been established; and no validated clinical trial endpoints exist [32]. Internal penetrating and perianal disease is mainly a challenge in CD. Limited descriptive studies implicate cytokines, such as IL-13 or the process of epithelial-to-mesenchymal transition in its pathogenesis [35]. The success of anti-inflammatory therapies is limited or recurrence rates are high [35].

There is a need to delineate early pathogenic events to elucidate intervenable factors prior to the establishment of fibrosis and which genetic or epigenetic markers could be the indicators/ triggers of these changes. There is also a need to increase the effort for the study of pathogenic factors of fibrogenesis, which include luminal (e.g. microbiota, host microbe interactions), extraluminal (e.g. mesenteric fat), and systemic drivers (e.g. fibrocytes or bone marrow derived stem cells).

E. Biologic determinants of disease location

The determinants of disease location for Crohn’s disease (CD) and ulcerative colitis (UC) along the horizontal axis of the bowel remains a major gap in knowledge. Two clinical models serve as surrogates for addressing this question, providing insights that may be relevant to pathophysiology events that determine IBD location. The first is the development of pouchitis in up to half of UC patients who undergo ileal anal pouch anastomosis within two years of creation of the J-pouch. Non-IBD patients who have
familial adenomatous polyposis rarely develop this condition, suggesting that pouchitis in UC patients recapitulates some aspects of the original disease. However, because ulcerative colitis is confined to the colon, it has been unclear why these processes now involve the ileal mucosa of the pouch (sparking the regions proximal to the pouch). A plausible explanation is provided by a prospective study that show that the UC ileal pouch undergoes changes consistent with colonic metaplasia, characterized by expression of colon-specific genes and decreased expression of ileal-specific genes [36]. These changes, which are observed in concert with the development of a colonic-like pouch microbiota, could represent a "hardwired" response unique to UC that is not observed in normal or FAP subjects. Many of the altered genes also involve inflammatory pathways and those associated with extracellular matrix remodeling and changes in the tight junctions as occurs in UC. The shift from ileal to colonic gene expression pattern in UC patients may be permissive for the development of subclinical inflammation. The development of frank clinical inflammation may be a consequence of a second set of triggering events, perhaps provided by regional gut microbiota. In monoassociated germ-free IL-10 deficient mice, for example, different commensal bacterial species selectively initiate immune-mediated intestinal inflammation with distinctly different kinetics and anatomic distribution in the same host [37]. These data suggest that the types and functions of regional gut microbiota and their effects on site-specific host immune responses can be important determinants of location, nature, and severity of mucosal inflammation. The mechanisms underlying these events are not understood and require further investigation.

The clinical heterogeneity of Crohn’s Disease also provides insights that may be relevant to disease location. Using a molecular stratification approach using RNASeq and chromatin profiling, a recent study mapped the levels of gene expression in non-inflamed, healthy-looking colon tissue samples taken from Crohn’s patients [38] and found that adult patients with CD clearly segregated into two classes based on colon tissue gene expression—one that largely resembled the normal colon and one where certain genes showed expression patterns normally specific to the ileum. These classes were supported by changes in gene regulatory profiles observed at the level of chromatin accessibility, reflective of a fundamental shift in underlying molecular phenotypes. Furthermore, gene expression from the ilea of a treatment-naive cohort of pediatric patients with CD could be similarly subdivided into colon-like and ileum-like classes. The former was associated with patients with rectal disease, or that required a colectomy. The latter primarily was characterized by an upregulation of pathways involved in lipid and xenobiotic metabolism, paralleling findings reported in the UC pouch study [36]. Although the sample size was small, the ileal subtype of CD was associated with patients who tended to have ileal disease that required intensive medical therapy. Despite these insights, the specific mechanisms or factors that determine where IBD occurs in the bowel remain unknown.

F. New therapies and response to treatment.

Animal models are used to explore mechanisms of action and provide proof of concept for therapeutics in a number of diseases including IBD. However, the unique interaction in the gastrointestinal system between host genetic and environmental factors makes modeling IBD in surrogate species challenging [39,
Even well-established models of colitis are plagued by reproducibility concerns across labs, which partially can be explained by differences in the microbiota present in different facilities [41]. Nevertheless, preclinical mouse models of inflammatory bowel disease remain a workhorse for mechanistic discovery of IBD pathophysiology and evaluation of therapeutics. There are numerous mouse models of IBD ranging from chemically induced colitis models to genetic models to models which are induced by manipulation of the immune response or addition of specific bacteria. However, while there are many in vivo models of IBD, none adequately predicts response to therapeutics. Every in vivo model has its strengths and weakness and the key to robust scientific investigation remains the selection of the correct model for the scientific question under inquiry with focus on mechanistic questions rather than direct translatable to efficacy in the clinic. The technical simplicity of dextran sulfate sodium (DSS) administration in the drinking water and the injury and inflammation it causes has led to a multiplicity of peer-reviewed publications that have not lead to direct patient benefit. In particular, surrogates of therapeutics which show benefit in patients including anti-TNF and anti-IL-12/23p40 antibodies are generally not efficacious in chemically-induced colitis models including DSS. This is likely because these are acute injury models run over a few days rather than a continuous disease process which develops over many years in a genetically susceptible individual. In other models, like the T cell transfer model where naïve T cells are transferred into a lymphopenic mouse (Rag null or SCID mutant mice), anti-inflammatory therapeutics are extremely beneficial. However, while mice fairly uniformly respond to a given therapeutic, there are no treatments which will adequately treat all individuals with IBD, demonstrating the need for multiple models to probe different aspects of the human disease. Understanding which models adequately represent different aspects of the human disease is key to understanding when and how to use one model over another to address appropriate mechanistic questions related to pathways and therapeutics. Additionally, mice with genetic mutations that confer significant and often severe immunodeficiencies have led to great insight into the immunopathogenesis of IBD; however, discoveries in such mice have only led to a handful of treatments that help a fraction of the over two million people afflicted with IBD in the U.S. with millions more worldwide. There is a need for models which are able to predict and explain the mechanisms behind failures in IBD treatments. One recent example is the unexpected failure of anti-IL-17 antibody to show benefit in Crohn’s disease, and in fact to lead to disease exacerbation in a subset of patients. A similar result was found in the Mdr1a mutant colitis model in which genetic deficiency in the P-gp protein leads to an epithelial barrier defect. In this mouse model, IL-17 inhibition lead to exacerbation of colitis and thus provided a model system to explore the effects observed in the clinic [42].

One of the important issues behind the limited translational application of experimental findings in mice into successful therapies for humans is the lack of models enabling study of human cells and the direct testing therapeutics rather than relying on surrogates which are rodent cross-reactive. Two recent advances in this regard are the development of humanized mouse models which allow the direct testing of therapeutics on human immune cells which are engrafted into immunodeficient mice and the use of explants.
or lamina propria mononuclear cells isolated directly from human IBD patient intestinal tissue both of which enable investigators to test therapeutic hypotheses directly on human cells and/or tissue [43, 44].

Lastly, current therapeutics are only efficacious in a subset of patients. Understanding which patients will respond to individual therapeutics is crucial to the advancement of treatment options and preclinical models which can identify the underlying cause of disease and the ability to predict who will respond to what type of therapy are required.

**Steps forward**

Several common themes have emerged in reviewing the current state of knowledge of preclinical mechanisms of human IBD. The existing research gaps in understanding the basic mechanisms underlying the pathophysiology of human IBD fall into several categories, as described above. To address these gaps, we propose both intermediate and longer-term approaches. Overall, to focus on preclinical research relevant to human IBD, we must prioritize both the use of patient samples as well as more appropriate models that better reflect the complexities and the temporal relationships of disease mechanisms that take place in human IBD. One successful approach for this is reverse translation: laboratory-based research addressing observations from clinical practice and/or clinical trials that in turn generates solutions to improve patient outcomes. A key feature of this approach is that it is initiated by using data obtained from patients with IBD. The key is to use an initial sample size that is robust enough to generate firm hypotheses that can be further tested. That being said, smaller, longitudinal, highly curated cohorts resulting in meta-analyses of patient-derived data can often be highly informative when patients serve as their own controls. In these subjects, recent ’omics technologies applied to clinical samples have produced an abundance of molecular and cellular data including patient genetics (through identification of susceptibility genes), microbiome surveys, and abnormal cell function as well as alterations of gene expression/protein function either for individual or groups of genes. Additional prospective longitudinal human studies, focused on specific disease entities or subtypes (ulcerative proctitis, UC patients with J Pouch-anal, Crohn’s patients with ileal resection at time of initial surgery that includes formation of ileo-colonic anastomoses) incorporating multi-omics analyses, in which patients can act as their own controls, and in which more complete metadata is obtained, could lead directly to predictive markers of disease progression. In addition, these data, and the novel systems approaches to synthesize connections between them can provide an overall hypothesis and guide for subsequent mechanistic work using animal models and in vitro approaches.

Essential characterization of normal cellular and physiologic processes is lacking and is needed to advance the field, including elucidation of the developing and aging immune system in humans and animal models; the processes of wound healing; the normal host-microbe interaction that selects for the regional microbiome; the normal microbial architecture of the GI tract (not just presence or absence, but also specific location of microbes with respect to each other and the host epithelium and the variation depending on region of the GI tract); the microbial-driven metabolome; immune-microbiota interactions that drive
homeostasis and colonization-resistance; and investigation of the non-bacterial microbiota-including viruses, bacteriophage, fungi, and archaea to determine their physiologic and immune impact. These fundamental areas of investigation will provide critical groundwork for understanding these processes during disease.

As the understanding of IBD pathogenesis and research on novel therapeutic and diagnostic approaches have evolved, novel models will need to be developed in order to enable adequate examination of these new aspects of biology as well as therapeutic modalities. New models are also needed to develop deeper insights into therapeutics targeting diverse aspects of the disease including strategies to shift the microbial composition, cell-based therapeutics to regenerate the epithelium or reset the immune response, metabolic manipulation, and cell-specific delivery to target therapeutics only to the cell of interest. Additionally, new models are needed to test the efficiency and specificity of targeting diverse pathways using new therapeutic modalities, including antisense oligonucleotides and cell-based and live microbial-based therapeutics. Models in which the human microbiome can be adequately grown, tested and manipulated in vitro and in vivo in the context of human immune and epithelial cells as well as the proper gastrointestinal environment including oxygen levels, pH, tension, etc. are urgently needed. In vitro advances in gut-on-a-chip technology as well as growth of human organoids in three-dimensional and air-liquid interface cultures show promise for development of such a system. Addition of the human microbiome from IBD patients into humanized mice in the context of colitis could enable testing of therapeutics targeting intestinal dysbiosis or microbial factors in vivo.

Development of preclinical models which will enable use of patient blood/tissue/cells to explore the individual’s underlying issue and cause of disease would greatly facilitate development of personalized solutions. This type of model could be based, for instance, on injection of patient-derived organoids into the mouse colon in a humanized mouse system. Additionally, focusing on reverse translational research to work from the human disease and observations in the clinic back to the lab will enable researchers to determine how to model diverse aspects of the disease or particular subsets of the disease to facilitate development of novel systems to enable testing of new therapeutic strategies.

The establishment of validated in vitro models is also a priority. The recent development of new models, including human-derived organoids, enteroids, and polarized epithelia that recapitulate the complex mixture of epithelial cell types in the GI tract, have been an exciting advance. Further advancing these models to include an immune cell component and potentially a microbial component could further the applicability of these models, both for studies of disease mechanism and therapeutic response. The plethora of approaches for the generation of these in vitro/in silico systems is a concern, leading to conflicting results and poor reproducibility. The establishment of consistent and validated methods to generate these models will allow rapid advancement in this area.

Improved and validated animal models that better represent human IBD are essential. These could incorporate single or multiple genetic polymorphisms, humanized microbiota and/or humanized immune
systems could be used to recapitulate the gene-environment interactions that lead to the development of IBD, and be used to understand disease progression, triggers of disease flares, and prediction of disease trajectory and response to treatment. Knocking out or knocking in mutations in specific cell types and sublineages would allow the investigation of the role of host cell intrinsic defects. The development of additional tools to specifically target cell lineages and sublineages is an immediate and essential first step to advance these studies.

Currently available animal models such as SAMP1/YitFc mice that develop CD-like ileitis spontaneously may be useful in understanding the cellular and molecular mechanisms and their temporal relationship in the development of intestinal fibrosis and stenosis [45]. Further clues regarding fibrogenesis could be derived from exploring underlying mechanisms of transmural disease in CD versus disease restricted to the mucosa and submucosa in UC, a phenomenon that could be explained by genetics, microbiota or differential inflammatory response. The observation that fibrosis can progress despite proper medical therapy and mucosal healing emphasizes the need to focus on inflammation independent mechanism of intestinal fibrosis, such as matrix mechanoproperties, matrix composition or matrix bound enzymes.

To study the effects of non-epithelial factors from other mucosal cells on intestinal epithelial cell function and wound healing, development of novel experimental platforms that allow co-culture of intestinal cells with immune cells and microbial cells. Such platforms will allow for the interrogation of many key questions such as the effect of inflammation that occurs in IBD on specific epithelial lineages. A goal is to define these interactions in terms of specific immune lineages that impact key epithelial functions (for example tuft cells and ILCs). How do these interactions affect epithelial and immune cell functions? Are the effects durable (perhaps through epigenetic modification)? We will also be able to interrogate the effects of alterations of the microbiota (bacteria, viruses, parasites) or microbial products that are known to occur in IBD on intestinal epithelial cells. The goals will be to determine the effects on IEC lineage function (direct or indirect).

Finally, despite the rapid evolution of DNA sequencing and ‘omics technologies for human and experimental samples, a major bottleneck to the field has been the analysis, integration, and interpretation of large datasets. Higher resolution visualization platforms and better genetic annotations for taxonomical and functional assignments are needed to better understand biological significance and impact. These sources of data have their own inherent noise, bias and limitations, confounding methods to integrate them. Better analysis tools capable of jointly accounting for these nuances are needed along with high-quality, well-designed experimental studies that combine these data sources. Thus, inclusion and partnerships with data scientists and experts in computational modeling and simulation are needed.

Human studies are inherently difficult due to large individual differences, challenges in controlling retrospectively for confounding variables, and constraints in observational clinical study design. However, with well-designed longitudinal studies that use multi-omic analysis interpreted in the context of clinical
metadata, tremendous insights into disease mechanisms, course, and outcomes can be gained. Coupling such insights with experimental models that involve patient-derived cells and other informative in vitro and in vivo approaches shows great promise ahead in making discovery-based advances that will greatly impact patient management and outcomes in IBD.

Filling the gaps in understanding the preclinical human mechanism of IBD will broaden our understanding of disease and accelerate the development of better classifiers for the clinical heterogeneity of IBD, recognizing individuals at risk and triggering factors that lead to disease, and advancing novel effective and targeted interventions for prevention and treatment of IBD.

CHAPTER II: ENVIRONMENTAL TRIGGERS

The etiology of IBD has been extensively studied. However, the causative factors are not fully understood. The importance of genetic susceptibility has been established in the last decade and genetic risk variants have been identified, but the lack of complete gene penetrance and the rapid rise of IBD incidence in certain geographic regions suggest that the interaction between genetic and environmental factors contributes to IBD. Several environmental risk factors including exposure to drugs (such as antibiotics), viruses, psychological stress, air pollutants, diet, and chemicals have been explored, but the data are still inconclusive. The main direction in this priority area is to move from association to causation through the following methods: 1) cohort-based epidemiological studies of exposures; 2) identification and validation of signatures of biological response to exposures; and 3) mechanistic studies of how environmental factors impact disease processes. Finally, it is critical to link these research approaches to clinical outcomes, to conclusively define how environmental exposures affect disease onset, progression and response to therapy. The current knowledge, the research gaps and the potential approaches to address these gaps are described below.
A. Epidemiology of Exposures and Disease Evolution: Concepts in Study Design

Two fundamental questions about CD and UC that have yet to be answered are what causes the disease and, once the disease is in remission with medical or surgical therapy, what causes the disease to relapse. In general, IBD is thought to result from one or more environmental exposures in a genetically predisposed host. These environmental triggers allow the genetically predisposed immune dysregulation to cause an aberrant response that ultimately leads to persistent bowel and in some cases extra-intestinal inflammation. More than 200 genetic risk loci have been identified, few of which have a strong association with IBD. Rather, the contribution of the genetic loci is thought to be less than 50% with the effect being greater in Crohn’s disease than ulcerative colitis [46]. Indeed, the rapidly increasing incidence of IBD associated with industrialization, first observed in Western societies and now being observed in many Asian countries [47], supports this notion. Thus, there remains great interest in understanding the role of environmental risk factors.

Environmental risk factors of great interest include, but not limited to, the following: 1) tobacco smoking, the most well-established environmental risk factor for Crohn’s disease and a protective factor in most studies of UC; 2) diet, in part because it has also been used as a therapy; 3) dysbiosis of intestinal resident microorganisms and exposure to pathogens; 4) psychosocial stressors; and 5) medications such as NSAIDs, antibiotics and oral contraceptives [48]. To study environmental triggers in IBD in the context of the epidemiology of exposures and disease evolution, observational epidemiology and identification of the critical window of vulnerability is needed to allow properly defining the cohorts, to account for frequency, duration and extent of exposure. The methods of observational epidemiology and reverse-translational research have and will likely continue to be employed in attempting to answer questions about environmental risk factors. Before embarking on such studies, there are a few important issues that investigators need to consider:

1) **Presymptomatic changes leading to IBD:** Among important research questions is the question of “Where does health end and illness begin?”. In clinical practice, we define the onset of inflammatory bowel disease as the time when either symptoms start or the time when the physicians first make the diagnosis. However, in most patients there has been an even longer period with the disease before this even happened. For example, Colombel et al observed that abnormal antibody responses to antigens such as *S cerevisiae* (ASCA) were detectable long before the disease manifest in military recruits [49]. Moreover, given the genetic contribution to the diseases, one might ask if patients are actually born with the disease in a preclinical state, waiting to accumulate environmental exposures and perhaps epigenetic changes.

2) **Critical window of vulnerability:** Knowing when disease begins is tightly linked to the question of “What is the critical exposure period for environmental exposures to cause disease?”. This is a critical question in designing studies focusing on the etiology of IBD, the answer to which is not
entirely clear today. Indirect evidence from studies of immigrants suggest that early life exposures may be more important than later life exposures [50].

3) **Properly defining cohorts to study ET (environmental triggers) in IBD**: Similarly, one must think about the population to study to answer the specific research question to account for disease incidence in relationship to the frequency, duration and extent of exposure.

4) **Predicting and following disease activity**: Studying disease exacerbations or loss of remission is in some ways easier than studying new onset of disease. However, even this can present technical challenges. It can be difficult to determine when the disease is truly in remission and when it has relapsed. For example, is the patient who has gone from severe symptoms and colonic inflammation to being completely asymptomatic, with a few small colonic ulcers remaining, in remission? Likewise, how many ulcers after surgery does it take to say that the person has active disease? Obviously, if colonoscopy, MRI or CT scan is needed every time we want to determine if a patient has relapsed, that will not be feasible. Therefore, new non-invasive biomarkers that accurately assess disease activity are needed. This would advance the field both clinically and from a research perspective.

5) **Loss of response to therapy**: Not all reasons for loss of response are the same. The patient who has developed antibodies to a biologic drug is different from the patient who is resistant to the same biologic drug despite adequate drug levels.

6) **From observational to hypothesis-driven studies**: Important research needs in this area include designing hypothesis-driven validation studies to advance from epidemiological observations to validation of potential causal effects of high priority environmental factors. Despite the vast number of association studies, validation and mechanistic studies are lagging.

7) **High priority environmental triggers of IBD**: Since the data on the causal effect of environmental triggers in IBD are almost non-existent, it is important to focus on triggers with previously-established strong associations with IBD to ensure faster return on effort and patient-valued impact in the short-term future. These high priority factors include diet, the gut microbiota, viral exposures, smoking, antibiotics, fungi, and psychosocial stress. Identification of the biological signatures caused by exposure to these factors is needed to direct investigators towards mechanistic studies and development of novel interventions. Below is the description of the research gaps in a few of these areas.

**Psychosocial stress**: In the context of IBD, psychosocial stress represents a perturbation of the homeostatic state of brain gut microbiome (BGM) interactions in response to events in the environment. Stress-related perturbations include increased intestinal permeability, immune system modulation and modulation of microbial gene expression and virulence by luminally released norepinephrine [51]. Current literature suggests that it is the subjectively “perceived” stress, and not the objective number or severity of stressful
events that is related to frequency and severity of disease flares in IBD [52, 53]. The sensitivity and responsiveness to stress is determined by genetic and epigenetic factors on the stress system and different brain networks the latter including the epigenetic effects within the hypothalamic pituitary (HPA) axis in response to exposure to early adverse life events. There are well-characterized brain networks that play a role in the response to stressful life events, including the salience network (determines the subjective relevance of events for an individual), the stress network, and the central autonomic nervous system output. As described above, it is assumed through observations that psychological stress exacerbates IBD flares, but little is known about brain-gut communications and the molecular interplay between intestinal and neuronal molecules that may play a role in IBD and that may represent a biomarker of disease related clinical manifestation.

**Diet.** Another important exposure related to IBD flares is diet. There are at least two aspects of diet that may be involved in IBD flares: 1) the proinflammatory effects of a typical Western diet, which is low in fiber and high in animal fat, sugar, emulsifiers and other chemical additives used in processed food. In rodent experiments, such a diet, or its components is associated with gut microbial changes, a reduction of the intestinal mucus layer and resultant increase in intestinal permeability and activation of the gut associated immune system. Food allergens and food hypersensitivity are triggers of IBD development. Modern production practices afford opportunities to change not only the fundamental composition of food but also introduce many understudied additive and pollutants, such as pesticides, polychlorinated biphenyls, preservatives, and heavy metals, which may serve as triggers of IBD [54]. This could be extended to changing metabolites generated by gut microbiota in response to alterations in food composition and the introduction of various additives such as artificial sweeteners [55] and emulsifiers [56]. The impact of dietary factors and pollutants as an environmental trigger of IBD symptoms have been extensively reviewed [54, 57, 58] and will only be lightly touched in this section. Since dietary intolerance is a common etiological suspect of symptoms, many patients and physicians have taken food elimination or exclusion to the extreme with only limited evidence-based support, although more promising evidence has begun to emerge more recently [59]. An exception to this is the use of elemental and other defined formula diets with a significant amount of evidence for the treatment of Crohn’s disease has been published [59].

**Intestinal microbiota and pathobionts.** There is substantial data indicating that the resident gut microbiota (which includes bacteria, viruses, mycobacteria, and meiofauna including worms, protists and other organisms) is one aspect of intestinal biology that is influenced by the environment. The microbiome plays a substantial role in controlling normal function of the intestine and contributes to inflammation and systemic disease as
well as both Crohn’s disease and ulcerative colitis [60]. Much emphasis has been placed on the bacterial microbiome in part due to the ready availability of effective and inexpensive computational tools for analyzing the population structure of complex bacterial communities through analysis of 16S rRNA gene diversity. Computational tools for other components of the microbiome are less well established, but are being developed and have been successfully applied. These include analysis of the virome which is altered and has a relationship to the bacterial microbiome in IBD [61]. Animal studies provide overwhelming support for the regulation of intestinal inflammation by bacteria, but also show that other components of the microbiome including viruses [62, 63], protists [64] and fungi [65] fundamentally alter intestinal inflammation. It is very likely that additional associations between components of the microbiome and IBD will be discovered. It is also clear that interactions between different components of the microbiome have significant effects on intestinal biology [66, 67], further underlining the complexity of the relationships between the microbiome and IBD.

Several important factors limit the impact of this area of IBD research. First, most studies have been cross-sectional and merely identify associations between a component of the microbiome and disease; longitudinal studies have not defined associations between the microbiome and disease activity or causation. Second, mechanisms responsible for observed associations are poorly understood, although some information is emerging [68]. Lastly, lack of mechanistic insights into the relationship between the environment, the microbiome, and IBD have prevented the development of broadly effective therapeutic interventions that matter to patients.

**Dysbiosis of the gut microbiota.** Although there has long been an interest in single organisms that play a role in the pathogenesis of IBD, such as *Mycobacterium paratuberculosis* [69] and adherent-invasive *E. coli* [70], more recent evidence supports the notion and the overall alteration in the composition of the gut microbiota associated with IBD, known as dysbiosis, may be important in disease pathogenesis. Dysbiosis of the gut microbiota develops in response to the intestinal inflammatory response via the production of alternative electron acceptor facilitating anaerobic respiration with an outgrowth of facultative anaerobes such as enterobacteracea [71] or inducing oxidative stress leading to the predominance of aerotolerant bacteria taxa belonging to the Proteobacterium phylum [72]. In turn, studies in murine model systems suggests that the dysbiotic microbiota in IBD may play a role in the pathogenesis of disease [73] [74]), a notion supported in patients with IBD based on preliminary evidence that fecal microbiota transplantation (FMT) is efficacious in the treatment of ulcerative colitis after multiple inoculations [75] and that meaningful clinical responses have been observed in patients
with refractory IBD who have received a combination of multiple antibiotics. Nevertheless, despite substantial mechanistic data that a dysbiotic microbiota plays a role in murine intestinal inflammation [76] [74], much more evidence is needed to determine if these findings can be reproduced in human IBD. Such evidence may be forthcoming based on ongoing industry sponsored clinical studies testing the safety and efficacy of gut microbiota therapeutics for IBD.

*Pathobionts*. Bacterial, viral, parasitic infections have been proposed to act as a trigger for the initial clinical presentation of IBD, thereby suggesting a role in the pathogenesis of this disease [77]. Such organisms may enter human body through food and contaminated water. Many of these microbial organisms can reside in healthy individuals and elicit no adverse effects and yet, in susceptible populations the impacts are significant [78, 79], hence the term pathobiont. The differential susceptibility can in part be explained by genetics. Alternatively, the mucosal barrier, resident mast cells and other immunological response regulators can be important determinants for disease development, recurrence and progression. However, it is usually difficult to study the mucosal barrier and tissue organization at real-time and over a long period (i.e. a longitudinal approach). For these reasons, the use of surrogate biomarkers for patient stratification (e.g. susceptible versus resistant) are in great need.

There is evidence to support a role for the intestinal fungi (the mycobiota) in the pathogenesis of IBD. Glycoprotein cell wall components can activate components of the innate immune system leading to immune signaling via molecules such as CARD9, interleukin 17 (IL17), interleukin 22 (IL22), NF-κB, NFAT, and ITAM [80]. Evidence in murine models supports a functional role for fungi in the pathogenesis of IBD and a comprehensive review on this topic has recently been published [81]. For example, CLEC7A knockout mice have increased susceptibility to chemically induced colitis due to their altered responses to indigenous fungi and a polymorphism in the gene encoding dectin-1 is associated with a severe form of UC in humans [65]. Observations from mice parallel the role for fungi in the pathogenesis of IBD in humans. Antibodies against *Saccharomyces cerevisiae*, a marker of CD, react with mannan, a yeast cell wall polysaccharide [82]. Multiple studies have shown that patients with IBD have alterations to the gut mycobiota; increases in specific fungal taxa have been associated with bacterial dysbiosis, increased human DNA in feces, and antibiotic use [83]. Furthermore, a recent study reported expansion of *Candida tropicalis* in patients with CD, and that levels correlated with those of anti-*S cerevisiae*, *E coli*, and *Serratia marcescens*; these 3 markers formed enhanced biofilms in mucosal samples patients with CD [84]. Finally, there is
preliminary evidence that the anti-fungal agent fluconazole reduces inflammation mice with colitis and patients with IBD [85].

**Pollutants and toxicants.** Our water and air are increasing polluted by various toxicants that are potential triggers for IBD. This is especially true with introduction of thousands of new chemicals to our environment annually with few being tested thoroughly by the National Toxicology Program for safety. Additionally, chemical pollution of air and drinking water associated with incomplete fossil combustion and chemical-laden release from hydraulic fracking are of concerns. Particular matters, NO\(_2\), SO\(_2\) and other related pollutants derived from incomplete combustion including air pollution and smoking can penetrate the GI tract barrier and cause oxidative stress, DNA damage and immune responses leading to IBD [86-89]. Aging municipal water supply infrastructure that laden drinking water with heavy metals, and the high frequency of radiation pollution of food and water sources from failed nuclear plants (Chernobyl and Fukushima) have been added to the long list of unknown, under-characterized, potential environmental triggers of IBD. Many of these have significant impacts on the immune system of vulnerable populations. Epidemiology studies have demonstrated that early-life exposure to radiation may be associated with IBD in children [90].

Tobacco is one of the sources of toxicants; however the effect of smoking on IBD is a focus of ongoing investigation. The robust relationship of smoking and IBD has been demonstrated across geographic populations. Moreover, this epidemiologic finding shows opposing effects between CD and UC, with smoking being protective against UC while being a risk for developing CD. Indeed, this may be one of the strongest pathogenic differentiators between CD and UC described. In UC, a systematic review and meta-analysis demonstrated a 3-fold increase in lifetime odds in non-smokers compared to smokers. Ex-smokers also have an increased likelihood of developing UC [91]. Smoking is not only a risk factor for the development of CD, but now numerous studies confirmed that smokers have increased risk of flares, surgery and postoperative recurrence [92]. Likewise, the odds of these complications seem to decrease upon smoking cessation suggesting that this is a disease modifying intervention [93]. A recent publication identified 64 SNPs for which the association between the SNP and IBD were modified by smoking behavior. Indeed, functional gene smoking interactions were suggested in two models of experimental colitis where genetic deficiency in IL-10 and NOD2 were also identified as potential human smoking-interaction genes in this study [94]. Nonetheless, major knowledge gaps exist in understanding basic mechanisms of smoking effects in terms of gene-smoking-immune system-microbiota interactions, and the opposing epidemiologic findings in CD versus UC.
B. Signatures of Biological Response to Exposures: The Exposome

To better understand the link between environmental exposures and disease, we need to identify the signatures of exposure, namely, measurable indicators of biological response to environmental factors, which can serve as biomarkers of disease onset, disease progression and response to therapy. Currently our knowledge of such metrics, i.e. genetic, epigenetic, metabolic, immunological, microbiological and other molecular signatures is very limited.

Current understanding of complex disease indicates that no single exposure can fully explain the etiology and natural history of a disease. The effects of exposure/triggers are often multifactorial, often occurring as combinations of endogenous triggers and multiple external environmental triggers including diet, chemicals and psychological stress. Unfortunately, the majority of epidemiologic studies in IBD have only examined the impact of a single chemical/trigger on disease development and outcomes [95]. This approach does not reflect the real-world relationship between often interacting exposures to multiple factors and disease development. A more accurate descriptor of environmental triggers for disease development is the concept of the exposome [96-99] which can be defined as the cumulative or acute impact of all the exposures of an individual in a lifetime and how these exposures interact with the individual's genome to elicit or prevent the onset, recurrence and/or progression of a disease. According to this concept, exposures at early-life [100] or other windows of vulnerability, as well as exposures to a combination of triggers/agents concurrently or in certain temporal sequences, are crucial determinants of disease onset and outcomes [101, 102]. In this regard, the exposome concept goes hand in hand with the notion of genetic heterogeneity in supporting a precision medicine approach to treat and prevent human diseases [96, 97]. However, the implementation of such an approach requires significant advancements in our ability to measure accurately multiple exposures as well as all components within the exposome. Methodologies to detect the interactions and to integrate information across the exposome-disease interface are still lacking, particularly for complex diseases and specifically for IBD. In connection to this research gap, identification of multiparametric signatures of exposures is needed to provide a full picture of exposome in IBD. As noted above, a recent study of primary sclerosing cholangitis, which is comorbid with IBD, has been funded by NIH to explore exposomic and genomic triggers. This can be exemplified by nutrigenomics studies, focused on different phenotypic response to diet, depending on the genotype of each individual [103]. In this context, nutrigenetics investigates how genetic susceptibility factors interact with environmental factors, including the diet and gut microbiota, and identifies the signatures of exposures to a nutrient or diet based on comprehensive whole genome analysis and microbiome-based data [104]. Moreover, given the interpersonal variability integrating multiple signatures, such as genome sequences, clinical tests, blood metabolome and proteome, fecal microbiome, as described by Price et al., (PubMed: 28714965), can be used to generate correlation networks and provide stronger evidence of the causal relationship between the exposure and the biological responses.
Given the complexity of the exposure and history of exposure new methods for measuring biological consequences in response to these exposures present a major challenge in the field. As such the challenge is to be able to analyze the massive amount of data coming from mega-omics analyses of target tissues (biopsies) to gain a better understanding of the etiology of the disease as it related to the complex triggers and trigger-trigger interaction. Thus far, only a few classes of endogenous and exogenous triggers related to IBD have been studied [52-54, 57, 58, 86-89, 105]. New bioinformatics and statistical methods for studying mixtures are needed to advance the field.

C. Mechanisms of How Environmental Exposures Drive IBD

To build the research continuum and advance towards diagnostics and therapeutic interventions, we need to validate the outcomes of the newly identified candidate signatures through investigating of the mechanisms by which environmental exposures can lead to perturbations in biological processes believed to play a role in IBD. For this purpose we need relevant model systems (animal models or patients).

Multiple models are available to study IBD related molecular networks. These include genetic models such as IL-1 mutants, chemically induced IBD models (DDS) and intestinal organoids, etc. To study the mechanisms that connect environmental triggers and IBD relevant model systems (animal models or patients) should be used to test a specific hypothesis. Studies are needed to establish the mechanisms by which combination of genetic risk factors and environmental triggers lead to disease (computational prediction models and animal models). Development of better animal models is needed to study mechanisms by which environmental exposures could impact remission, disease flares, complications and response to treatment. Finally, a reverse-translation approach, that starts with clinical observations of vulnerability to exposures during disease development or over the course of life, should lead to hypothesis-based studies that can be implemented in the laboratory to identify the underlying biological changes related to disease onset and progression in response to exposures.

Steps forward to advance the understanding of environmental triggers in the pathogenesis of IBD

A. Comprehensive characterization of the exposome in IBD

The exposome is defined as the totality of human environmental exposures from conception onwards [96, 97, 106]. In this regard, the concept that IBD is yet another complex disease that originates from early-life [107] should be considered. In this context, the window of vulnerability can be extended from prenatal [108, 109] to pre-conception period [110] and other developmental stages including puberty, pregnancy and aging. If new onset IBD in adults is due to exposures that occurred in early childhood, the feasibility of such studies retrospectively will be extremely limited. In the short term, research looking at preclinical immune dysregulation, preclinical microbiome changes, preclinical serology, etc. could help define the preclinical latent phase of inflammatory bowel disease. To perform these studies, it will be critical to acquire human samples in well-designed epidemiology studies that allow for gene by environmental trigger studies to be...
performed by taking advantage of technological advances in deep analytics to define new signatures for precision medicine IBD. Such studies may provide much needed new insights into the role that environmental factors play in disease pathogenesis. The broad sharing of data generated from epidemiological, clinical and experimental studies is highly desirable to leverage the IBD communities' investment in these studies and advance the field of environmental trigger for the disease. Collectively, these new initiatives are aimed at advancing precision medicine-based disease risk assessment, monitoring, prevention and therapies. The overall objectives for the next five years could be accomplished through: 1) expert consultation services in medicine, epidemiology, exposure science, and statistics; 2) sharing resources of cohorts with environmental exposure data and biorepositories; 3) building a core of expertise in analysis of exposure mixtures; 4) informatics tools and 5) “Integrative Thinking” transdisciplinary workshops that will mesh information from various disciplines for translation to the bedside and precision medicine for IBD.

Since resources are always limited, priorities should focus on the “priority” cohorts to study the signatures of environmental exposures. These may include patients with high frequency of flares and those with persistent symptoms after mucosal healing. It is now well-recognized that chronic low-grade exposure may elicit responses drastically different from those caused by acute exposure; and both might lead to development of common complex diseases. There is mounting evidence indicating that the lag time for disease development, especially for low-dose exposure, could be very long. In some cases, it may extend out several decades, which is the basis for the emerging field known as “Developmental Origins of Health and Disease (DoHAD)”.

It is important to realize that individuals or populations are not equally endowed. Marked genetic variations predispose their unique susceptibilities to a particular exposure. Hence, the interplay between genes and exposures, window of susceptibility, life-style or life-stages modifiers, and their complex interactions are all crucial data gaps to be filled. With the completion of the Human Genome Project and twenty years of Genome-wide association studies (GWAS) the next challenge resides in defining the influences of specific environmental factors on the epigenome(s), which pivotally control which part of a genome will be expressed, in a specific cell/organ, along critical developmental time times. The complexity of these interacting forces of nature makes environmental health/medicine complex and challenging.

To fill these knowledge gaps, multidisciplinary systems biology approaches using multi-omics platforms on human biospecimens tied to impeccable clinical databases will advance the IBD field. Analyses of these gargantuan data sets require sophisticated informatics and machine learning. Special attention has been paid to early origin of disease, windows of vulnerability (prenatal, in utero, puberty, pregnancy and old age), the interplay between genetics and epigenetics, genetics, epigenetics with other omics (proteomics, metabolomics, metagenomics, and metallomics), the influences of life style modifiers such as diet or stress on epigenetic reprogramming, and finally the data void related to continuous life-long editing of early developmental program.
Ultimately, future directions for IBD research focused on the exposome include:

(1) Expansion of comparative population-based exposure studies focusing on regional versus international cohorts. For example, an attractive way to assess the impact of stressful life events on IBD outcomes would be to study a large number of patients (1,000+) with frequent online sampling of subjective symptoms (online questionnaires), stool and blood samples for gut microbial metabolites and for stress-related changes. There are now commercial entities that can perform such large-scale studies enrolling patients online almost exclusively relying on the assessment of ambulatory questionnaire and biological information. Out of the collected data, applying machine-learning approaches, it is possible to extract meaningful correlations and causal relationships between stressfully perceived life events and various biological outcomes.

(2) Emphasis on emerging environmental triggers (e.g. nanomaterials, triggers related to climate change related disasters). Untargeted metabolomics and exposomics are a promising strategy to capture information about emerging or unknown environmental triggers.

(3) Development of ambulatory, wearable systems to continuously monitor responses to exposures in real life situation. In terms of stress, there are several reliable wearable devices which can assess the impact of stress on an individual. Approaches that are being evaluated include ambulatory assessment of heart rate variability/vagal tone, ambulatory assessment of skin conductance (sympathetic nervous system activity), sleep patterns, relative time spent in bathroom, physical activity.

(4) The development of novel statistical and bioinformatics methods for analyzing, identifying and mathematically modeling the individual component and/or interactive effects of a multiple exposures. For example, it is now possible based on results from mechanistic studies in animals and large studies in humans to create mathematical (systems biological) models of the circular interactions between the brain, gut, and gut microbes. Feeding biological data into these systems makes it possible to quantitate the impact of life events perceived as stressful by an individual with increased stress responsiveness on biological readouts such as mucosal inflammation, pain and discomfort etc. Multi-omic analysis is being addressed by many research groups. It is critical not to reinvent the wheel for IBD, but rather to partner with collaborators with similar interests.

(5) Emerging new focus on mitochondrial genomics and epigenomics to better understand maternal “inheritance”,

(6) Continued acceleration of application of artificial intelligence to multiparametric analysis of exposome-derived data and related clinical metadata;
Platforms to study single-cell ‘omics signatures to uncover mechanisms underlying heterogeneity in cellular and personal responses to environmental perturbations. Collectively, these new initiatives are aimed at advancing IBD trigger research towards precision medicine-based disease risk assessment, monitoring, prevention and therapies.

B. Considerations of study cohort design for the characterization of the exposome in IBD

One can divide populations into those with a high or low incidence of IBD. A potential advantage of a high incidence population is the ability to identify very high incidence subpopulations and follow them prospectively. In contrast, if one is designing a population-based study in a high prevalence region, it is possible that ubiquitous risk factors with small effect sizes have contributed to the high prevalence and the ubiquitous nature of the risk factor will make it difficult to detect the association. In contrast, in areas where the incidence of IBD is just beginning to rise, one could speculate that the key environmental risk factors may also be rising as well and that there would be less universal exposure, therefore increasing the ability to detect the association with new onset IBD. Groups with potential high yield for studying etiologic factors in disease incidence include: 1) children under age 10, which are a population with rising incidence rates even in developing countries [31], because their lives are less experienced and easier to track with parents included to help map what has happened to them; 2) the elderly who like have different phenotypes and different risk factors for IBD than children; 3) individuals living in rapidly industrializing societies such as those in Asia including China and India, and 4) immigrants. Example frameworks for such studies are depicted in Table 1.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Design</th>
<th>Clinical data collected</th>
<th>Biosamples collected</th>
<th>Follow-up period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children of parents with IBD</td>
<td>Prospective cohort study</td>
<td>Diet history every 3 months in first year, every 6 months in year 2, and every year thereafter; Infection and drug use including antibiotic use from birth one every 6 month review; Psychological surveys, health surveys every 6 months</td>
<td>Blood at birth and at one year and annually thereafter; Stool samples every 3 months for 1 year and then annually;</td>
<td>Birth to age 18</td>
</tr>
<tr>
<td>Patients over age 65 newly diagnosed with IBD and control patients without IBD</td>
<td>Case-control study</td>
<td>Diet history prior to diagnosis; Other lifestyle factors prior to diagnosis; Medication history prior to diagnosis; Psychological surveys at diagnosis; Infection history; Tobacco use</td>
<td>Stool and blood samples at diagnosis and index date for controls</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>
Immigrants newly diagnosed with IBD and immigrants without IBD

| Case-control study | Breastfeeding information from birth (from parents);
| | Infection and medication use including antibiotic use from birth;
| | Psychological surveys at diagnosis;
| | Tobacco use;
| | Age at immigration;
| | Region of immigration;
| | Living conditions before and after immigration

Stool and blood samples at diagnosis and index date for controls

Studies seeking to understand the role of the environment in underlying biology of IBD through studying relapse of disease need to consider why patients have relapsed. As such, not all patient groups will be equally informative. Here, we propose a few populations that may lend themselves to use in studies of environmental risk factors for disease flare. These include patients who have undergone a surgery for Crohn’s disease, patients only taking mesalamine or diet-based therapies given the non-immunosuppressive nature of these therapies, and those patients in deep and stable remission who elect to discontinue their medical therapy.

There should be an emphasis in studying the interactions between multiple exposures, or the temporal sequences of exposure to major triggers, an approach which traditionally has not been the focus of IBD research which has focused on single types of exposures. For example, psychosocial stress, dietary factors, food additive and pollutants have all been shown in rodent models to affect intestinal permeability, intestinal immune activation and microbial composition and function. In humans, the genetic heterogeneity presumably plays an important role in determining which patient will develop IBD or experience a flare to a single category of these exposures, and in which patients a combination of exposures is required to result in symptoms. The lack of information or study focus is due in part to the lack of methodologies to tease out the individual of combinatorial effects of the exposures and their biological outcomes in animal models or in patients. To identify both the impact of an individual trigger, as well as the potential associations of multiple triggers, and to characterize the interactions, and cumulative effects of multiple triggers, is critically needed to understand the role of environmental triggers in the etiology of IBD.

For example, there are several ways of quantifying the biological response to stress in humans:

- Multimodal brain imaging, assessing the engagement of different brain networks to a laboratory stressor, and the altered responsiveness and structure of relevant brain networks.
- Measurement of HPA axis and ANS responses to a laboratory stressor
- Assessment of gut microbiome and stool metabolite changes in response to stress (reduction of lactobacilli, changes in tryptophan metabolites)
Assessment of intestinal permeability and systemic immune activation in response to chronic stress,

Untargeted metabolomics to capture network level changes in stress-related biochemistry

The traditional target measurements may not be informative for the treatment of patients as individuals. The identification of “fingerprints” or unique signatures associated with exposures which can predict disease development and evolution in vulnerable individuals will advance the field. In this context the use of unbiased multi-omics approaches as read outs will help patient stratification and individualization of treatment regiments. This requires promotion and fostering of integrative transdisciplinary thinking and approaches in environmental triggers of IBD among clinicians, epidemiologists, and basic scientists. A system biology approach that integrate multiple platforms of signatures including genomics, epigenomics, proteomics, metabolomics and microbiomics with exposomics as readouts of disease states associated with environmental triggers is an opportunity that can individualize patient treatment and management. An important limitation to such data driven approaches is the required sample size (1,000+) and to a lesser degree computing power.

Extensive medical records, dietary recalls, ambulatory stress assessments (using mobile apps), and spectrometry-based exposome measurements [111] during different stages of disease evolution are needed to construct a comprehensive platform for integrating the omic-based readouts of environmental triggers for IBD. Significant advancements in qualitative method development including novel statistical and bioinformatics methods, machine learning, data integration and visualization and new tools in clinical informatics will be the absolute necessity for realizing the power of omic signatures for any disease including IBD.

C. Moving from association in the exposome to causation.

From a disease management point of view, it is not always possible to assess the target tissues, hence evidence-based determination of what kind of tissues or cell types could be used as surrogate tissues (e.g. blood cells, buccal cells, fecal cells, urine) for characterizing flares, remissions, and drug responsive and non-responsive, with high concordance will forge the field forward. In studying the mechanisms we need to address what are the cause-and-effect and whether this can be reversed. Understanding these relationships may elucidate the course of biological events that lead to various clinical manifestations and may help to address our challenge of having a heterogeneous cohort. Future studies should focus on the elucidation of how exposures to environmental triggers could lead to alterations in IBD relevant functions and its relation to particular clinical outcomes. Optimally, subsequent to the identification of environmental signatures associated with IBD, studies should optimally be designed to determine the mechanism(s) by which these exposures to play a role in in the pathogenesis of IBD. No doubt that these studies will be challenging to conceptualize, design, and execute in humans. One approach might involve small cohort studies, with high stringency analytics to identify the right computational tool to further study host response
mechanisms. Such an approach might be integrative in nature ultimately requiring validation in a larger cohorts. High dimensional and biostatistical approaches, possibly involving deep learning and artificial intelligence, may play a role in helping to connect the mechanism to environmental signatures. For example, such approaches may help integrative analysis combining genetic risk factors and environmental triggers to identify potential cross-talk among the biological pathways affected by both factors. When identifying protective factors studies could involve both human investigation and animal models. Longitudinal sample collection should be performed in animal models and validated in humans to identify the critical windows of vulnerability and the long terms effect of one-time verses persistent exposure. Finally, from a technical standpoint in culture-based and animal model systems, Crispr/Cas9 technology for gene editing performed in existing animal models and in organoid systems may allow to introduce multiple genetic factors and superimpose that with the effects of environmental triggers.
CHAPTER III: NOVEL TECHNOLOGIES

In parallel with the need for advances in understanding the biology of IBD, there is equal need for new or improved technologies to serve clinical needs in IBD. These advances may be particularly impactful in three areas of need: 1) non-invasive modalities to detect and monitor active inflammation related to IBD and assess treatment response; 2) technologies to enable mucosal targeted drug delivery systems that enhance efficacy and decrease side effects; and 3) surgical and other technologies to prevent post-operative septic complications, treat fistulizing disease and prevent anastomotic failure.

A. Non-invasive Detection and Evaluation of Active Inflammation in IBD : Imaging and Sensing Technologies

Evaluation of CD activity and treatment response remains a particular challenge. Current clinical assessment of disease activity in adults and children relies heavily on clinical scores such as the Crohn’s Disease Activity Index (CDAI), which is based on patient documentation of symptoms over a 7-day period. Another scoring system – the Harvey-Bradshaw Index is an abbreviated clinical scoring system that is based only on patient symptoms at the time of evaluation [112]. Moreover, there is sometimes a disconnect between patient symptoms and measures of disease activity. Circulating biomarkers of inflammation such as erythrocyte sedimentation rate (ESR) and c-reactive protein (CRP), as well as fecal biomarkers, calprotectin and lactoferrin, are used to track disease activity in a quantitative manner but are not specific to IBD-related inflammation and also cannot evaluate changes in inflammation at the bowel segmental level [113]. Disease monitoring using non-invasive sensing technologies able to detect changes in IBD-related clinical parameters (e.g., intestinal inflammation, mucosal healing, changes in microbiome, dysregulation of immune system) could be particularly useful for early recognition of disease activity in asymptomatic patients and for monitoring of response to therapies.

In the absence of optimal non-invasive monitoring technologies, endoscopic measures of disease activity (and treatment response) remain the gold standard for inflammatory activity because of their ability to assess mucosal inflammatory changes in individual bowel segments. Mucosal healing, defined as resolution of endoscopic inflammatory changes in a bowel segment following treatment, has recently been established as a therapeutic endpoint associated with long-term clinical remission. The advantages of endoscopic indices include: (1) direct visual assessment of disease severity, (2) ability to follow individual bowel segments over time for changes in disease severity, and (3) ability to perform endoscopic mucosal biopsy to obtain microscopic evaluation of disease. However, endoscopy has several limitations; (1) invasiveness including requirement for patient sedation/anesthesia, (2) financial cost, (3) inter-user variability and limited ability to precisely localize the bowel segment being evaluated, (4) lack of established cutoff values to define response/remission [114], (5) the technical challenge associated with reaching remote intestinal segments such as jejunum or proximal ileum or intestine beyond a stricture segment.
These limitations constitute major impediments to the frequent use of endoscopy to measure treatment response in routine clinical practice.

Radiological imaging, due to its inherent non-invasiveness, makes a practical modality for the detection and monitoring of CD if other limitations could be overcome. Currently ultrasound (US), Computed Tomography (CT), and Magnetic Resonance Enterography (MRE) are commonly used for CD evaluation. Each technique has advantages and limitations. Conventional ultrasound is comparatively inexpensive, does not use ionizing radiation and can be performed in real-time with easy accessibility. Conventional ultrasound has been shown to be very effective in the monitoring of patients with CD, particularly in the pediatric age group. However, ultrasound is operator dependent and subject to inter-operator variability. In addition, interpretation is qualitative further increasing operator dependency. Finally, it is difficult for ultrasound to evaluate the entire length of large and small bowel as a screening tool and may miss extra-luminal disease complications due to acoustic attenuation from air within the bowel lumen.

CT directly demonstrates bowel wall thickening, mesenteric edema, and lymphadenopathy, as well as inflammatory masses and abscesses. CT is not effective in detecting mucosal inflammation and radiation exposure prevents its frequent use particularly in the pediatric age group. Like CT, MRE has cross-sectional imaging capability that enables it to evaluate extraluminal and extraintestinal disease manifestations of IBD, an important advantage over endolumenal techniques. With the addition of oral contrast distention of the bowel and intravenous contrast delineation of bowel vascularity and inflammation [115], MRE has been shown to be highly accurate for the detection of active CD in both adults and children [116]. Over the past five years, MRE has become the primary imaging modality for disease assessment in patients with Crohn’s disease.

Due to their limitations (invasiveness, cost, ionizing radiation, discomfort, limitation in the evaluation of the entire gastrointestinal tract), these tests are reserved during episodes of symptomatic exacerbation. With the advent of molecule-targeted biologic therapies, which are expensive and have potential side effects with long-term use (e.g., infection, lymphoma), there is a need for imaging techniques that can provide early and frequent assessment of treatment response. This would allow a temporal window for dose escalation or addition of combination therapy to maintain clinical remission. Further, early detection of non-responsiveness would help to minimize the toxicity and financial cost associated with non-effective medications.

Another challenge is the evaluation of strictures and the evaluation of the length of bowel involved by CD. Strictures can be inflammatory (which are typically treated medically), fibrotic (which are typically treated mechanically), or mixed [117]. Radiological imaging does not perform well for stricture evaluation because imaging signs of active inflammation can obscure underlying fibrosis, while endoscopic imaging and biopsies only sample the mucosa so are not deep enough to detect submucosal and serosal collagen deposition indicative of fibrosis. The length of involved bowel is one factor to be considered when making
decisions regarding additional medical therapy or surgical treatment. When a recurrence occurs after resection of a long segment of bowel it is usually as long. If additional resections are necessary, patients are at high risk for short gut syndrome [117].

B. Technologies to enable mucosal targeted drug delivery systems that enhance efficacy and decrease side effects

About one-third of these patients require surgery within 5-10 years of diagnosis, and two-thirds of patients require surgery over their lifetime [118]. Although hospitalization rates have decreased in the biologics era, several publications have shown that rates for surgery have not significantly decreased [119]. After surgery, the majority of patients go in remission, but the disease almost always recurs over time, and in general at the surgical anastomosis. Most studies have reported that endoscopic and histologic recurrence precedes clinical recurrence [120]. The early mucosal lesion seen in Crohn’s disease - the aphthous ulcer - can be seen by visualizing the ileum proximal to the anastomosis by colonoscopy within months after surgery. Endoscopic disease has been shown to guide risk for clinical disease and risk of re-operation. It is estimated that 70-90% of patients will have endoscopic disease within 1 year of surgery and this number increases to 80-100% within 3 years. Clinical recurrence is seen in up to 30% of cases in one year. About one-third of the patients will require reoperation in 10 years and 80% in 15 years [120]. Risk of reoperation is significant as loss of small bowel is not compatible with healthy lifestyle as it leads to malabsorption and in select cases, short bowel syndrome.

One of the causes of disease recurrence could be ineffective drug delivery or medicine non-adherence. Medical treatment for IBD is lifelong thus requiring patient compliance for disease-free remission. Medication non-adherence remains a barrier to effective treatment in IBD [121]. More infrequent dosing has been demonstrated to increase adherence in chronic conditions [122]. To address the need for more effective drug delivery, to achieve reduction of unwanted side effects associated with systemic therapies, and as such to increase treatment adherence, multiple approaches of localized drug delivery and sustained local drug release are currently emerging. Additionally, since it has been observed that recurrent disease occurs in the majority of cases at the site of the anastomosis and in the bowel segment proximal to it, these new approaches to localized and sustained drug release may offer possibilities to intervene locally at the time of surgery or postoperatively with endoscopy in this region. Improved mucosal drug delivery systems targeted to this area of highest recurrence risk could enhance efficacy and decrease side effects.

C. Surgical and other technologies to prevent post-operative septic complications, treat fistulizing disease and prevent anastomotic failure.

Septic Complications: Septic complications in IBD occur predominantly in patients with a fistulizing phenotype of CD and in patients who have a complicated course after IBD surgery. Both conditions affect the quality of life and disability of the patient and are a great burden on health care resources [123].
**Fistulizing Crohn’s disease of the perineum:** Perianal CD affects one out of four patients with Crohn’s disease. To find better solutions to manage fistulae-in-ano in CD, it is important to understand its pathophysiology. The pathogenesis of a fistula-in-ano starts with a deep penetrating ulcer responsible for a bowel wall perforation resulting in an abscess, which in turn perforates spontaneously or is drained surgically through the perineal skin. The resulting fistula persists as long as the internal fistulous opening remains patent due to active inflammation and chronic low grade sepsis along the fistulous tract. The pressure gradient from the diseased anorectum to the perineal skin during defecation aids in the patency of the fistula. Management of the fistula should be directed at healing of the anorectal mucosa, at closure of the internal fistulous opening and at improving the healing environment in the fistulous tract through appropriate drainage of perianal sepsis. Much literature supports this approach [124]. Biologics have shown promising results but their discontinuation is followed by resumption of drainage. Surgical techniques alone aiming at closure of the internal opening, for example, simple closure, mucosal advancement plasty or ligation of the intersphincteric tract have proved to be disappointingly ineffective.

**Anastomotic failure:** The Achilles heel of IBD surgery is anastomotic failure manifesting as intra-abdominal abscesses and peritonitis. The impact of this complication on the patient and health care resources is enormous. Prolonged hospital stays, reoperations with stoma creation, the development of entero-atmospheric fistula and incisional hernia have profound effect on the quality of life of the patient and cause great disability to take part in normal life.

The cause of anastomotic leakages is multifactorial. Some of the known factors can be corrected (malnutrition, treatment of preoperative septic conditions, weaning of medications associated with a higher risk of anastomotic dehiscences); some strategies can be employed (pre-ablitation or use of staged surgical procedures), but other conditions cannot be modified (male gender, comorbidities, high content of visceral fat, deleterious side effects from chronic use of steroids and biologicals).

Despite all efforts, and in the presence of a well vascularized anastomosis, without tension and with a perfect apposition of the two ends providing an “air and water tight” seal, patients still suffer anastomotic dehiscence rates between 2-23%, depending on the site of the anastomosis [125]. Why a seemingly perfect anastomosis still can leak is unclear and poorly understood. Improvements are needed in prevention, early detection of leaks and consequently more effective management of dehiscences.

**Steps forward**

**Non-invasive technologies for evaluation of active inflammation in IBD.**

There is a pressing need to use novel imaging or a combination of existing imaging technology to address the gaps noted in detecting and monitoring disease extent and activity. Regarding detection of active disease, multiparametric MRI is one such approach. Newer techniques such as Diffusion-Weighted MRI
DWI and motility imaging have shown promise. DWI hyperintensity correlates well with endoscopic inflammation in CD, but the correlation is less than that for ulcerative colitis. DWI has an advantage that it can be used in unprepared bowel segments (i.e., no IV or oral preparation), which suggests that it may be used in imaging of patients for whom IV contrast administration is contraindicated or who cannot tolerate oral bowel preparation [126]. Motility imaging of the small bowel can be performed with cinematic thick slab steady state free precession sequences, which allow repeated acquisition of images to visualize bowel peristalsis. These sequences allow both qualitative and quantitative assessment of bowel motility. Bowel motility imaging can be very helpful to distinguish under-distended bowel from abnormal bowel in patients with poor oral contrast intake for MRE. In CD, abnormal bowel segments have altered motility [127]. In essence, a combination of DWI, motility imaging and MRE performed potentially as low-cost, rapid acquisitions can provide a multiparametric MRI technique that can address some of the need gaps highlighted above.

Multiparametric ultrasound is another combination approach that holds promise. While conventional B-mode ultrasound has several advantages due to being real-time, it does not provide additional information in terms of early markers of inflammation or fibrosis. Ultrasound Shear-wave Elastography (SWE) is a novel quantitative ultrasound imaging technique that allows anatomic mapping of tissue elasticity using shear waves to evaluate underlying tissue stiffness, making evaluation non-operator dependent and quantitative. This technique has shown promise in quantitative assessment of fibrosis in the liver and early studies show that inflammation and fibrosis in the bowel also have a signature. Furthermore, extent of inflammation can be quantified using advanced Doppler Techniques and contrast ultrasound techniques. These advanced Doppler techniques in combination with contrast enhanced ultrasound are recent technological advancements in the field of ultrasound imaging. These techniques have several advantages over conventional Doppler imaging: (1) low velocity flow visualization, (2) high resolution, (3) minimal motion artefact, (4) high frame rates. These advantages allow micro-flow detection, the depiction of which is particularly useful for assessment of disease activity as a result of inflammation. The combination of conventional US, shear wave elastography, contrast ultrasound and advanced Doppler techniques offer the possibility of using the low-cost, readily available nature of ultrasound imaging to sequentially follow-up patients in remission and identify patients who do not respond to therapy early. These methods in combinations hold promise to address the gaps for imaging in Crohn’s disease patients. Robust clinical studies that can evaluate quantitative values differentiating inflammation and assessing grades of fibrosis need to be performed.

There are a number of additional imaging modalities that may be of benefit in the management of IBD. Video Capsule Endoscopy (VCE) has demonstrated advantages over MRE techniques in evaluating the proximal small bowel and has been shown to be highly sensitive for the diagnosis of mucosal lesions. The added advantage of VCE is that it can allow evaluation of both small bowel and the large bowel [128]. In addition, it can allow monitoring of patients in remission with very limited side effects. Additional techniques
that are newer but hold promise to address the gaps are Multispectral Optoacoustic Tomography and ultrasound molecular imaging. Multispectral Optoacoustic Tomography can be used to image structural features of tissue. Newer approaches that can be performed trans-abdominally and can detect early inflammation can help fill some of the gaps. Ultrasound Molecular Imaging is still in the early phases but early studies show promise as targeted molecular contrast agents can adhere to areas of inflammation and fibrosis. These can use existing US systems but standardization of signals from contrast agents is still needed.

Development of new sensing technologies (biosensors) and integration of existing sensing technologies are needed to detect and monitor inflammation in the context of IBD, using existing and novel biomarkers. New sensor technologies should be capable to detect the signals related to biological processes regarded to be causative and/or highly correlated with intestinal inflammation in IBD (e.g., cytokine levels, presence of proinflammatory immune cells, proinflammatory mediators, etc.) and should be informative with respect to symptoms (e.g., ability to detect an active ‘flare-up’ prior to observation of severe symptoms, or the ability to distinguish between symptoms caused by inflammation and symptoms occurring in the absence of inflammation).

These biosensors, should be non-invasive or minimally invasive (implantable, ingestible, wearable or environmental devices or nano-sensors) and should provide real-time or near-real-time, continuous or periodic, data sampling and reporting, to be recorded together with the corresponding physiological relevant fluctuations in the specific biological signal(s). Such devices would allow routine monitoring of the disease during daily life without the need for a visit to a healthcare facility. Technically novel technologies, developed in an experimental setting should be practical and patient-friendly- with high potential for rapid approval for consumer market and cost-effective production.

An example of sensing technology that can be potentially adopted for IBD monitoring is intestinal gas capsule, which can sense variations in the luminal gas composition [129], particularly changes in hydrogen and hydrogen sulfide (H2 and H2S) that may be associated with exacerbation of IBD. Work is currently being conducted with engineered commensal bacteria and yeast designed to sense various mediators of inflammation. A “sense and respond” version can be envisioned where bacteria not only sense inflammation but are able to deliver an anti-inflammatory signal.

Lastly, newer technologies are being investigated that would allow capsules to sample mucosa or luminal content for “omics” analyses as well as deliver drug to specific targets of the GI tract [130]. This line of research is promising.

**Targeted drug delivery systems**
The observation that recurrent disease frequently occurs at the site of the anastomosis and in the proximal bowel segment after a resection and anastomosis offers the possibility to intervene locally at the time of surgery or postoperatively with endoscopy on this region. Drug diluting stents could be possibly inserted at the time of surgery or endoscopically at the time of detection of early recurrence. These stents could be “refilled” by oral administration of drugs that are designed to attach on the stent to achieve maximum concentration on the segment at high risk for recurrence.

Another approach is to harness inflammation-targeting drug delivery to achieve high drug concentrations locally at the site of inflammation with minimal exposure of healthy or distant tissues. Inflammation-targeting hydrogel (IT-hydrogel) microfibers, prepared from generally-recognized-as-safe agents, can selectively adhere to the inflamed tissue in the gut and release the encapsulated therapeutic agent on-demand in response to inflammatory enzymes, including MMPs at the target site [131]. IT-hydrogel microfibers loaded with dexamethasone (Dex) demonstrated preferential adhesion to inflamed epithelial surfaces in two different mouse colitis models in vivo and to inflamed lesions in colon tissue samples (ex vivo) from patients with ulcerative colitis. Dex-loaded IT-hydrogel enemas administered every other day to mice with colitis resulted in a significant reduction in inflammation and 5-10 fold lower peak serum concentration of drug compared to free Dex enema. In addition to advancing potentially safer steroid based therapies to rapidly achieve remission with reduced systemic side effects, the IT-hydrogel approach is amenable to oral administration for potential treatment of Crohn’s disease and the platform can accommodate a wide spectrum of therapeutic agents including hydrophobic/hydrophilic small molecules and biologics. This technology is currently being tested.

Lastly, gastric resident systems capable of residing safely in the stomach for several weeks and providing extended drug release have been described [132]. Further investigation and extensive clinical research are needed to assess the effectiveness of local delivery using existing IBD therapeutics.

**Technologies to prevent and manage post-operative septic complications, treat fistulizing disease and prevent anastomotic failure.**

As a first step towards making progress in the treatment of perineal septic complications in CD, there is a need for a proper classification for fistulae-in-ano that combines characterization of the size, pathology and shape of the tracts, as well as disease activity at the mucosal level. Similarly, to vet the results of different treatment modalities and interventions, it is necessary to identify proper endpoints including the impact of the fistula on the patient’s quality of life.

Technology aiming at surgical closure of the internal opening to transiently reduce inflammation to permit wound healing to occur, as well as technologies for sealing of the fistulous tract should be developed and explored. There is a need to develop surgically inserted scaffolds carrying anti-TNF’s or stem cells that are released in the tract to simultaneously close the internal opening and improve the healing environment.
The ideal scaffolding fills the tract initially with a material that promotes healing after which it will be fully integrated or dissolved by tissue that replaces it. To address this need scaffolding technology and biocompatible sealants are currently being developed to be used as an enhancement for tissue regeneration at the site of surgery and potentially to be used as a therapeutic option for post-surgical complications and fistulizing disease [134].

In order to make progress in the rate of anastomotic healing, additional studies are needed on the optimal bowel preparation prior to surgery [135]. Proper vascularization of bowel ends depends on good surgical technique and experience and is traditionally assessed subjectively. New techniques using fluorescent technology can probably better assess the vascularization of the bowel ends particularly in minimal invasive surgery where imaging is required. Other technology should focus on tissue sealants aiming to reinforce the anastomosis and making it perfectly “air and water tight”. Most of the anastomoses currently are made using mechanical stapling devices. A drawback of the stapling devices is that the staple heights are constant while the tissue thickness varies. There is an unmet need for “smart” stapling devices adapting the staple height to the thickness of the tissue of both bowel ends to have the optimal tissue compression combining a robust adaptation and “air and watertight” seal while preserving vascularization between the staples. New technology should focus on methods to improve and accelerate wound healing at the site of the anastomosis reducing the initial phase of physiological decrease in anastomotic strength because of collagen breakdown and build up. Recently, there has been great interest for the causative role of a collagenase producing microbiome that might be involved in anastomotic leakage [136]. New interventions targeted on this microbiome could potentially reduce the rate of anastomotic dehiscences.

It is clear that if a leak is going to happen, the earlier the diagnosis is made the better the outcome of a more timely intervention. The assessment of CRP after surgery has shown of great benefit. A low CRP at day 4 has a great negative predictive value and precludes septic complications reliably [137]. High CRP levels at day four should drive aggressive imaging to rule out or diagnose anastomotic failure. Early management of anastomotic failure before the development of septic problems is key to preventing the full sequel of events and is the best prevention of worse outcome. Technology focusing on sensing an imminent leak before day four could further improve the management of leakage. Smart sensors might have a role in this.

CHAPTER IV: PRECISION MEDICINE

Overarching Goals

The overall goal of precision medicine in IBD is to utilize specific clinical and biologic characteristics of individual patients to predict the disease course and tailor treatment in delivering optimal care. While studies over the past decade have begun to address this goal, knowledge gaps remain in terms of precisely
which clinical and biologic factors will provide the greatest utility in predicting disease course and assessing clinical outcomes in response to therapy over time. Regulatory and economic barriers must also be considered. Steps forward will require study of ongoing, and newly refined cohorts that include children and adults, with integration of clinical and deep molecular phenotyping data across studies, and development and validation of biologic signatures in the context of these cohorts. These data will enable future studies that will test the clinical utility and cost-effectiveness of biomarkers in the clinic. Ideally this work will also be adopted earlier in drug development programs, to ensure that as new medications come to market these can be used in a more targeted way.

Adopting a precision medicine approach in IBD requires a better understanding of the impact of genetic and environmental factors on efficacy of different treatment regimens. As an example, an emerging area in cancer treatment strategies includes treating cancer patients with a combination of drugs based on a systems biology analysis of tumor gene expression data in the presence or absence of pharmacological perturbation in experimental systems [138]. This approach, which is currently being tested in various settings, will revolutionize pharmacotherapy.

Expanding on a recent commentary on the prospects and challenges of precision medicine approaches in IBD [139], three priority areas were identified:

1. Studies of disease susceptibility, activity, and behavior;
2. Prediction of drug response;
3. Optimizing current and developing new molecular technologies to enable precision medicine

A. Understanding the natural history of IBD: Disease susceptibility, activity, and behavior

The clinical course of Crohn’s Disease (CD) and Ulcerative Colitis (UC) is quite variable. A number of recent pediatric and adult-onset cohort studies have sought to define factors associated with a more aggressive course, with the implication being that “high risk” patients would benefit from earlier introduction of therapies that alter natural history including biologic therapies [140]. We also need to identify patients with relatively low risk of experiencing poor outcomes, in order to inform their risk/benefit analysis. For example, this could reduce the exposure to unnecessary and costly immunosuppression with biologics in the low risk population.

Prospective and Inception Cohorts. Inflammatory bowel diseases (IBD) affect an estimated 1.6 to 3.1 million Americans [140, CDC link]. Practical challenges in managing patients include variability in disease presentation, response to therapy and long-term outcomes among patients with IBD. There is increasing recognition that IBD may represent a group of common pathways of multiple disease mechanisms that lead to chronic inflammation of the digestive tract, and in some cases, extra-intestinal organ systems. In order to provide precision medicine to patients with IBD, we need to be able to better define patient subsets at
different levels risk of developing aggressive disease course and who are likely to respond to specific therapies, and so gain knowledge on how to change the natural history of the disease.

The availability of large, well-characterized longitudinal cohorts that include patients with IBD across the life span, from very early onset IBD to late onset IBD can help to meet these challenges. We have learned from RISK [30], PROTECT [141] and other cohort studies (see below) that enrolling patients with IBD into prospective cohorts is feasible, but that successful projects require substantial funding, committed leadership, oversight and high enrollment (to capture small but important clinical subgroups). These studies also underscore the need for long-term follow-up to understand how current and future therapies affect the natural history of IBD. The ability to perform human translational studies in RISK and PROTECT has been instrumental in advancing our understanding of and how different disease mechanisms produce distinct clinical phenotypes (e.g., stricturing vs. penetrating Crohn’s disease) [30].

**Very Early Onset IBD (VEOID):** Recent studies have reported that from 10% to 20% of children diagnosed with IBD at a very young age, currently defined as < 6 years of age, may in fact have a monogenic form of disease [142]. This includes classical primary immune deficiencies such as chronic granulomatous disease (CGD) presenting with gastrointestinal involvement, as well as auto-inflammatory disorders due to loss of IL-10 signaling. A diagnostic algorithm including functional immune testing and targeted gene sequencing panels has entered the clinic for this population, with results guiding in some cases curative stem cell transplantation. This represents the best example to date of a precision medicine approach in IBD, albeit in a focused population of VEO cases.

**Pediatric IBD:** The Crohn’s & Colitis Foundation-sponsored RISK CD inception cohort study enrolled over 1000 children at diagnosis at 28 sites in North America and sought to define factors associated with progression to complicated disease phenotypes (stricturing or internal penetrating) within five years of diagnosis. A model for disease complications was developed and validated, which may be applied in clinical practice [30]. Children with older age-of-onset, African American race, and ASCA IgA and CBir1 sero-positivity were shown to be at higher risk for stricturing and internal penetrating disease complications. For the first time, the added value of pre-treatment ileal gene expression signatures was demonstrated, both in terms of highlighting key pathogenic mechanisms, and patient risk stratification. Validation of these gene signatures is ongoing. A comparative effectiveness analysis of early anti-TNF therapy within the RISK cohort demonstrated higher rates of year-one steroid-free remission, and reduced progression to year-three internal penetrating complications, in those who received anti-TNF within three months of diagnosis [143] [30]. A similar National Institutes of Health (NIH) sponsored PROTECT UC inception cohort enrolled over 400 children at diagnosis at 24 sites in North American, with predictive models for steroid-free remission and need for rescue with anti-TNF or colectomy anticipated in the coming year [141]. Collectively, these studies have provided an approach to identify pediatric CD patients at highest risk for disease complications, and for the first time, a window of opportunity to intervene with early anti-TNF therapy.
**Adult-onset IBD**: The adult-onset CD IBSEN and CONNECT cohort studies have also defined factors associated with a more aggressive disease course over the first five to ten years after diagnosis [144]. Age at diagnosis (<24 y.o.), jejunal disease location, and structuring or penetrating disease behavior and perianal lesions at diagnosis were identified as risk factors, with higher association with poor prognosis. In the IBSEN study, age at diagnosis, need for systemic steroids and ASCA serology served as a basis for 5-year risk prediction model, with the probabilities for advanced disease ranging from 8.6% to 92% [145]. The adult-onset UC IBSEN cohort study has defined factors associated with risk for colectomy [146]. These include age at diagnosis, disease extent, need for systemic steroids, and CRP/ESR inflammatory markers, with probabilities of colectomy ranging from 2.6% to 40.1% based upon combinations of predictors at diagnosis. A SNP-based genetic risk scoring system showed the ability to divide adult-onset UC patients from low to high risk for colectomy, with probabilities of 0%, 17%, 74% and 100% in the four groups [147].

A peripheral blood CD8+ T cell transcriptional signature distinguished both CD and UC patients who would experience a frequently relapsing course [148]. The PROFILE study is currently ongoing to evaluate utility of this non-invasive prognostic signature to deliver personalized therapy in CD, and improve clinical outcomes. Most recently, a prospective CD cohort study, PROSPECT (Personalized Risk and Outcome Prediction Tool), has sought to define the clinical utility of models based upon clinical, demographic, genetic, and serologic factors. A novel system dynamics analysis (SDA) methodology has been applied, together with development of a tool to present results to patients in fostering shared decision making. Completion of PROSPECT will provide critical evidence for the clinical utility of risk stratification in practice.

**IBD PLEXUS**: IBD Plexus, named for its complex network of parts, was founded by the Crohn’s & Colitis Foundation in 2015 with a mission to advance science, accelerate progress towards precision medicine and improve the care of patients living with IBD. The national-scale, cloud-based platform integrates clinical, patient-reported, genomic and molecular data from diverse patient cohorts. IBD Plexus provides academic and industry researchers with access to research-ready datasets and analytical tools to more rapidly perform activities that promise to speed treatment development, and optimize existing therapies through the use of biomarkers and diagnostics, and improve health outcomes. In 2017, IBD Plexus launched with over 60 academic institutions / medical centers and seven pharmaceutical members. SPARC IBD – Study of a Prospective Adult Research Cohort with IBD – is a multi-centered longitudinal study of adult IBD patients, which is collecting and linking clinical data, patient-reported outcome data, and serial biosamples through the course of the patient disease. Data and samples are available through IBD Plexus for basic, translational and clinical research with the goal of finding predictors of response to therapy and predictors of relapse that will lead to precision medicine strategies and new therapeutic targets. When fully enrolled, SPARC will follow 7,000 well-phenotyped adult patients.

A comprehensive list of existing IBD cohorts is provided in the supplemental materials. While these cohorts vary in their goals, sizes, characteristics, and data definitions, there may be an opportunity to leverage the existing data and samples to yield additional significant research findings. Importantly, a better
understanding of current patient cohorts will reveal limitations in their scope and design to identify future opportunities.

B. Prediction of drug response

Because the anti-TNF class is currently the most effective approach for CD and UC, with widespread use, this section will focus on how to predict response to that class of medications.

Several studies have tested the role of perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) in predicting response to anti-TNF in UC. Overall, patients who are negative for pANCA were found to have higher rates of response to anti-TNF [149]. This may guide more aggressive dosing of anti-TNF in pANCA+ UC patients, or alternatively earlier switch to alternate biologics [150].

A few small studies have explored the utility of proteomics in predicting anti-TNF response, with some candidate proteins reported [151, 152], but no large-scale validation has been performed. Additional studies have suggested the promise of gene expression signatures to predict anti-TNF response in CD and UC [153]. A study of colonic mucosal healing identified a gene panel with high accuracy for non-response to anti-TNF in CD [154]. The same group also reported a gene panel for prediction of non-response to anti-TNF in UC [155]. In a more recent study, patients with UC or Crohn’s colitis with the greatest overall disturbance in colon gene transcription were reported to be the least likely to respond to anti-TNF [156]. Oncostatin M (OSM) drives pro-inflammatory cytokine production in the gut. Consistent with previous transcriptional studies, patients with high levels of pre-treatment OSM have recently been reported to be less likely to respond to anti-TNF [157]. Validation of these signatures of anti-TNF non-response in additional pediatric and adult cohorts incorporating current therapeutic drug monitoring (TDM) practices would be of great value. However, data suggest that a more generalized signature for non-response, reflecting the highest levels of mucosal transcriptional dysregulation, may emerge regardless of the specific therapy tested.

Response prediction is currently hampered by a confluence of imprecision. Phenotype and definition of response and remission is currently a highly subjective endeavor. The commonly used scales of MAYO and CDAI have critical components that depend on qualitative input from patients and physicians. More quantitative measures such as mucosal healing and tissue state also require a significant interpretive element. This situation creates a level of imprecision that leaves response and remission ill-defined and produces data sets with high variance. In the context of a disease that waxes and wanes over time, with significant environmental effects, robust definition of positive response becomes difficult. We cannot predict what we cannot adequately define. To address the phenotype issue we need a molecular marker of disease state that eliminates the more interpretive aspects of response and remission definition. Such markers, or molecular disease profiles (MDPs) are in development for IBD.

Guided by the MDP development, companion diagnostics will enable more accurate drug selection and potentially decrease the risk of adverse effects. Companion diagnostics are needed to overcome the limitations of current clinical and endoscopic measures in positioning and monitoring of immune modulator,
biologic, and small molecule therapy. Exemplified by the RISK and Janssen PROgECT clinical studies, we need to expand MDPs to define response and deep remission and incorporate them into cohort studies and clinical trials.

In addition, we need to assess whether response to treatment depends on the state of the disease, for example, whether some patients are not responding because at the time of treatment they have too much disease drive or too much inflammation for the current agents to overcome. If this hypothesis were to be confirmed, then there would be a need for a predictive marker that would be disease state specific. This is supported by transcriptomic data linking the greatest overall gene expression dysregulation to the lowest likelihood of corticosteroid or anti-TNF response in UC [158].

Finally, we need markers of post-operative course in CD and pouch outcomes after IPAA to guide medical and surgical decisions for patients with early and late CD or UC with intact bowel.

In conclusion, despite an ever-increasing array of therapeutic options for medical management of CD and UC, biomarkers are needed to target therapies to specific patients and optimize their use.

C. Optimizing current and developing new molecular technologies to enable precision medicine

A number of studies have utilized genetics to try and define predictive response markers. After nearly 20 years since the NOD2 gene discovery and follow-up by highly informative GWAS and sequencing studies, we have not been able to define genetic markers of response. It may be time to conclude that classic heritability is not a major component of response to treatment and may be less important in disease predisposition and prognosis than initially hoped. Alternatively, precision medicine requires implementation of a systems biology approach that integrates multi-omics derived data for generation of predictive models of disease course and response to treatment [159].

There are currently various technologies that could contribute to this multi-omics characterization of IBD patients, to better understand the pathways contributing to IBD pathogenesis, and to determine the impact of therapies in IBD patients. These include the assessment of RNA (transcriptomics) and protein (proteomics) expression in blood and intestinal tissues. In addition to the direct \textit{ex vivo} measurement of RNA and protein expression, relevant perturbations of peripheral and mucosal cells can further highlight patient distinctions. DNA regulation is a critical determinant of RNA and protein expression, and both DNA sequence variant analysis through genotyping approaches (e.g., exome and whole genome sequencing), and epigenetic analysis, through DNA methylation, histone modification and ATAC-seq studies, can provide insight into mechanisms regulating DNA. Also, of tremendous benefit has been the ability to establish organoid culture systems, thereby enabling the dissection of differences in intestinal epithelial cell characteristics and modulated outcomes between individuals. CRISPR-Cas9 and other gene-targeting technologies have allowed for critical gene modulation in these organoid culture systems, as well as in a variety of other cells pertinent to IBD pathogenesis. This, in turn, has provided a means for clearly
establishing the roles of specific genes and the consequences of various gene variants in the cells examined, as well as the ability to restore function of IBD-relevant pathways perturbed in these cells in vitro. Finally, current technologies in microbial profiling from both stool and intestinal biopsies, including through 16s rRNA sequencing, shotgun metagenomic sequencing, metabolomics and culture systems, have enabled characterization of microbial communities and microbial functions in IBD patients, with additional dissection in the context of disease phenotype, genetic susceptibility, and both medical and surgical therapies.

As suggested by multiple studies, intestinal homeostasis is maintained though a balance of cells with different phenotypes, often with opposing functions. Thus, single cell profiling enables the identification and quantification of cellular sub-populations and allows to follow the dynamics of the phenotypic transformation as a function of time, location and biological stimulations. In a healthy organism intestinal homeostasis allows balanced regulation of immune activation and tolerance. In IBD, single cell changes may indicate the source of disruption in the homeostasis, at the inflamed mucosal site, in a structurally different tissue (e.g. fibrogenic) and throughout of different stages of maturation and migration. Studies utilizing single cell RNA-seq and ATAC-seq on cells from intestinal tissues are now underway as a means of defining what is anticipated to be an increasingly more complex spectrum of cell types contributing to IBD pathogenesis. Characterizing the spectrum of cell phenotypes through protein expression is similarly underway in suspended cell populations, for example utilizing CyTOF, and in tissue sections, utilizing approaches such as tissue imagine mass spectrometry (IMS) or multiplexed ion beam imaging (MIBI). Yet another area of development has been in T cell receptor sequencing and antibody epitope characterization, with advances in both these areas having the potential to provide additional insight into disease pathogenesis and into changes in these measures over the course of the disease. Also important in future advances is the refined implementation of various computational approaches, such as the development of improved deconvolution algorithms for extracting cell populations from whole tissue, novel algorithms for interrogating microbiome data, and technologies employing learning models to characterize findings in radiology, endoscopy and pathology in IBD patients.

**Steps forward**

Suggested approaches to address these critical gaps are described below.

**Studying disease susceptibility, activity, and behavior**

The value of robust longitudinal studies of adult patients with established disease has been identified. To further understand different aspects of the genetic, environmental and social impacts of IBD and how this relates to effective treatment, this type of data needs to be collected and accessed routinely by the scientific community. Standardization of sample collection, processing and analysis will be a key factor that will enable progressive data sharing and integration across research and clinical institutions, as well as unification of protocols and terminology. Ideally, this would establish a strong foundation for validation
studies in the context of well-defined IBD cohorts, for clinical studies to test the clinical utility and cost-effectiveness of biomarkers in the clinic and for drug development programs.

In general, the design, collection, storing, analysis, reporting and data sharing frameworks of bio-samples need standardization and harmonization at the global level for purposes of both transparency and accessibility. We should create a “Data Commons” with objective of pulling together the best “omics” datasets available, with the goal of enabling data access to and global collaboration. A data commons (and/or access to biobanked samples) would allow researchers to refine, replicate and/or validate disease findings and biomarker findings in independent cohorts. Ideally this infrastructure will allow access to data and biospecimens collected prior to and following treatment with established and novel therapeutics and to recruit and follow patients in a longitudinal manner from both academic cohort studies and industry-sponsored Phase III randomized clinical trials (RCTs).

One way to harmonize precision medicine approaches in the international scientific community is to provide and employ resources to optimize and harmonize research protocols and practices, considering consensus-based best practices for conducting IBD studies. Such measure could include generation and sharing of data dictionaries, software tools, study templates and data collection methodologies, following the example of the NIDDK IBD genetics consortium. The construction of an online analysis toolkit resource highlighting the most up-to-date methodologies adopted internationally in the clinical, statistical and economic areas and resources to online or academic short courses. This would include but not limited to study design, quantitative and qualitative analysis methods, outcomes analysis, decision analytic modelling, Bayesian tools for evidence synthesis techniques, validation of biomarkers, budget impact analysis, early health technology assessment (HTA), and techniques for reporting and dissemination to various stakeholders.

**Prediction of drug response**

Healthcare providers seek to improve risk stratification approaches to make informed decision on the course of treatment as well as be able to quickly detect the effect (or lack of such) of the prescribed therapy on the course of disease. In this context, one approach could be to focus on cohorts with extreme phenotypes. This will allow to make a clear differentiation between high and low-risk patients.

Building on the outcomes of published risk stratification studies, such as RISK and PROSPECT, we need to integrate pediatric and adult CD clinical and serologic models, utilizing Systems Dynamics Analysis (SDA) and other similar approaches to build a model for predicting complications/surgery [160] and test clinic-based shared decision-making tools to guide early introduction of anti-TNF therapy. Furthermore, studies are needed to validate the RISK ileal gene signatures as predictors of CD complications and the impact of early anti-TNF therapy. Validation studies of clinical, serologic, and genomic predictors of natural history in pediatric and adult UC cohorts, incorporating the impact of early anti-TNF in high risk patients (e.g. PROTECT), are also needed.
More qualitative research is needed at national levels to understand and address barriers to adoption of precision medicine in IBD. Research is also needed to address the economic barriers to implementation of precision medicine tools. To enable a better risk/benefit analysis to aid in choosing a drug, we should identify patients at high and low risk. Successful outcomes of such studies will satisfy the considerations of economics of development and implementation. In perspective, addition of precision medicine tools to the clinic would potentially enhance shared-decision making for timing of biologics and surgery, and thereby improve long term clinical outcomes while reducing costs. Follow-up periods are not typically long enough to mitigate costs of the diagnostic tool relative to standard of care.

There is also a need to explore this further and assess usefulness of precision diagnostics in the IBD care pathway. The generation of a roadmap to make stronger cases to support diagnostics within the current reimbursement framework is warranted given the high initial cost outlay of bringing new diagnostic tools into the care pathway. Validation studies of a companion diagnostic (e.g. through tissue transcriptomics) for prediction of anti-TNF response or non-response in pediatric and adult CD and UC populations will allow the field to move towards MDPs to define response and remission.

Finally we should encourage biomarker development within drug development programs, and active collaboration between pharma and academic investigators. These collaborations will be critical to developing clinically useful diagnostics to inform more precise therapeutic choices in the future.

Optimizing current and developing new molecular technologies to enable precision medicine

The primary approach for optimizing technologies and protocols for advancing precision medicine studies should be standardization of procedures related to data and samples collection, data repository and biosample banking should be implemented. In this regard, standardization of data dictionaries, data collection platforms and barcoding of biological samples should be adopted. In addition, to enable integration of clinical, serologic, and multi’omic approaches to predicting patient outcomes, we need to use state-of-the-art machine learning and systems biology approaches. Finally, we need to firmly establish the value of whole tissue versus single cell approaches. Using a bottom-up approach it may be necessary to identify markers from purified cell populations first and then work toward measurements from more accessible samples such as tissue biopsy or blood samples.
CHAPTER V: PRAGMATIC CLINICAL RESEARCH

RCTs have been considered the gold-standard of trial design in medicine since their implementation in the 1940s[161]. However, there are several key limitations of this study design[162]. Firstly, patients enrolled in RCTs may not be representative of real-world treatment populations due to the strict exclusion of specific subgroups: pre-defined criteria may limit enrollment for patients due to prior medication exposures, age, or surgical history. Particular patient populations may also not be interested in participating, due to the need to hold beneficial medications or concerns regarding randomization to placebo. This has particularly impacted RCT enrollment rates for children, who are more challenging to recruit. Multiple factors can also delay the publication of RCT results for several years, and the conduct of these studies can be quite costly. Lastly, RCTs focus on one treatment effect over a relatively short follow-up period as opposed to several possible clinical effects or treatment plans.

Over the past 15 years, there has been a rapid expansion of clinical resources available to researchers in medicine and epidemiology. Pragmatic clinical trials, which may be defined as trials which are intended to “inform a clinical or policy decision by providing evidence for adoption of the intervention into real-world clinical practice” [162, 163], and observational studies conducted with these resources have the potential to address many knowledge gaps in the understanding of the epidemiology of and management of IBD that have historically been challenging to assess through RCTs.[163] There are several advantages to these approaches. The use of real-world populations allows for the measurement of real-world medication effectiveness. With a wide array of patients enrolled in large populations, there will be increased heterogeneity, allowing for the evaluation of specific subgroups, including difficult-to-recruit
patient populations such as children or the elderly. Pragmatic and observational studies, conducted in the scope of routine care or with previously collected data, can be conducted at lower costs and produce findings more rapidly. Lastly, these studies have the potential to increase follow-up time, generating invaluable data on long-term medication effectiveness or rare adverse events.

However, there have traditionally been several barriers to conducting pragmatic clinical research in IBD. Firstly, many of the methodologies may be unfamiliar to researchers, who may not be aware of clustered randomization for comparing treatment effects among two medications, or statistical methods such as risk or propensity score matching to compare “like” individuals, thereby approximating RCTs[164]. Large datasets derived from claims-based data may not contain important confounders such as disease location, severity, or environmental factors. Linking datasets to include such variables has historically been challenging and costly.

Despite these barriers, there is a growing acceptance of these methods and findings. There have been several prominent naturalistic observational study designs in cardiology and neurology[165]. The Corticosteroid Randomisation After Significant Head injury, or MRC CRASH study, was a large, pragmatic trial with minimization of inclusion and exclusion criteria in order to maximize enrollment[166]. Similarly, the Thrombus Aspiration in ST-Elevation Myocardial Infarction in Scandinavia (TASTE) utilized simplified enrollment criteria, a large national registry, and limited follow-up to minimize barriers to enrollment to assess the impact of thrombus aspiration prior to percutaneous interventions in ST-segment elevation myocardial infarction[167]. The mHealth Screening to Prevent Strokes (mSToPS) trial combined direct-to-patient advertising, a large commercial registry, and minimal restrictions to providers caring for enrollees to assess a wearable technology for detecting atrial fibrillation [168, 169]. Two recent studies in IBD also used pragmatic, cluster-based designs. De Jong and colleagues used a clustered design to study a telemedicine system and healthcare utilization, while the Randomised Evaluation of an Algorithm for Crohn’s Treatment, or REACT trial, compared early combination therapy to conventional treatment.[170]

The addition of new research resources in IBD make this an ideal time to further employ pragmatic clinical research methods to improve the care we provide for patients with IBD. There are an increasing number of large, merged datasets capable of better capturing the continuum of care in patients with IBD. There is also increasing interest in validating existing datasets that have not previously been used to study IBD, such as the Center for Disease Control and National Center for Health Statistics’ National Health Interview Survey (NHIS), one of the nation’s largest national surveys of health-related data[171]. There have also been significant strides in the development of prospectively collected cohorts (TABLE X in Precision Medicine Section) with highly granular patient data, such as SPARC IBD, IBD QORUS, RISK, and IBD Partners. All of these resources will be invaluable for future IBD-related clinical research.

In this section, we highlight how pragmatic research methods can be used in the coming years to address several key clinical questions regarding the natural history of IBD and the clinical care we provide to patients affected by this disease. The main knowledge gaps that will benefit from a pragmatic clinical research approach were identified in the following areas: A) understanding the epidemiology of IBD; B)
A. Understanding the incidence and prevalence of IBD in the United States

The current incidence of CD in adults is estimated to be 6.3-23.8 per 100,000 person-years, while for UC it is estimated to be between 8.8-23.1 per 100,000 person-years[172]. Incidence rates have historically been slightly lower in pediatric cohorts in the United States (CD incidence: 0.66-8.8 per 100,000 person-years; UC incidence 0.34-5.3 per 100,000 person-years)[173]. However, recent systematic reviews in both populations have demonstrated that worldwide IBD incidence and prevalence are likely changing over time, with some countries experiencing continued rapid increases in incidence and others with stabilization of rates[172, 173]. In fact, a recent systematic review suggests that incidence rates in North American may be stabilizing, or even declining [172]. However, these findings are derived from only four studies conducted in California, Olmstead county, and Rhode Island [174-177]. In fact, a recent report by the Centers for Disease Control utilizing the CDC’s NHIS suggests the prevalence of IBD in the United States was 3.1 million individuals in 2015[178], up to 3-fold higher than previously published estimates[179][176][176][175][135]. As policy makers and funding bodies rely on these estimates, obtaining accurate data is important to ensure appropriate funding allocation.

Observational studies have the potential to address this knowledge gap in the coming years. Prior epidemiologic research in the United States has been limited in part by the lack of comprehensive, longitudinal datasets, compared to data derived from more comprehensive systems such as the Vision computer system in the United Kingdom or Denmark’s nationalized healthcare system [180]. This is in part secondary to the fragmented model of care delivered in the United States, with multiple private and federal payers, and a lack of a unified electronic medical record. However, merging of existing datasets may help to more comprehensively assess the shifting epidemiology of IBD in the United States.

Several existing datasets may assist in estimating IBD incidence and prevalence rates. For example, while the CDC’s NHIS has appreciated a significantly higher prevalence of IBD than prior studies conducted in Olmstead County or California [178], this survey-based dataset requires validation of IBD diagnoses to ensure they are accurate. Larger federal or commercially based claims datasets, alone or in combination, could also be used to confirm these estimates. Costs have historically been a barrier to these efforts. Additionally, each dataset may contain or lack specific coding, such as inpatient information or specific medication recording. These databases are also sensitive to patients entering or leaving due to insurance status or geographic relocation. However, as they often include tens of millions of patients, they could prove to be an important resource.

There have also been multiple recent advances in merging secondary datasets. Researchers in pharmacoepidemiology have recently created several distributed data networks, collating data from multiple sources to study rare diseases or rare medication- or device-related adverse events[181]. Examples of such networks include the Food and Drug Administration’s Sentinel System[182], the Accelerating Data
Value Across a National Community Health Center Network, or ADVANCE network[183], and National Patient-Centered Clinical Research Network (PCORnet)[184]. These networks can contain hundreds of millions of patient-lives. Significant data curation, including the development of common data models, ensuring anonymity, and data quality assessments are required in order to develop these “data lakes”, increasing the costs of these resources. However, these datasets have already allowed for assessments of the rare risk of intussusception with pentavalent rotavirus vaccination[185], comparing bariatric surgical approaches in pediatric patients[186], and assessing the appropriate aspirin dosing for cardiac event prevention[184]. There have been limited attempts to use such data in IBD, however. One particular example is the use of a distributed network to assess the incidence of IBD in pediatric populations in Canada. Using collated data from all 5 provinces in Canada, which typically employ separate data warehouses, Benchimol and colleagues were able to appreciate a rapidly rising IBD incidence in those <5 years of age[31]. Researchers should emulate similar efforts in both pediatric and adult patient populations in the United States.

B. Understanding how to more accurately select medication for patients with inflammatory bowel disease to increase treatment effectiveness

Over the past two decades, the available agents to treat IBD have rapidly expanded, progressing from anti-TNFs to anti-leukocyte adhesion agents, biosimilars, anti-IL-12/23 biologics, and most recently the small molecule tofacitinib[187-192]. These medical therapies still do not address all patient needs: Primary non-response rates to anti-TNFs range from 10 to 30%, while secondary loss of response rates are considerably higher, with up to 46% of patients experiencing recurrence of symptoms at the end of 1 year[193]. These estimates are potentially problematic, however, due to heterogenous follow-up periods during induction or maintenance phases, as well as different clinical, endoscopic, and adverse event-related outcome measures[194]. Even when considering these differences in trial design, clinical trials do not account for gaps between clinical trial efficacy and real-world effectiveness[195].

Therefore, clinicians are often presented with the conundrum of determining in whom we should use which agents as first-line therapy. As previously noted in the Precision Medicine section, there are limited data providing clinical guidance on this topic. Additional research is required to identify serologic, biochemical, or genetic markers that may predict which medications are ideal for specific patient subgroups. This is the unifying goal of personalized medicine, with fields like oncology at the forefront of applying these methods.

New, highly granular observational clinical research datasets, combined with advances in predictive modeling, may aid in helping to identify in whom we should initiate specific therapies. Current initiatives within the Crohn’s & Colitis Foundation will soon provide the large, comprehensive datasets required for this research. One such dataset is the Study of a Prospective Adult Research Cohort with IBD, or SPARC IBD cohort. This initiative is prospectively collecting highly detailed clinical and laboratory data, in combination with biospecimens, on up to 7,000 patients with IBD from a wide array of centers of excellence across the United States. These data will provide us with the opportunity to analyze an atypically large...
number of covariables to identify who might best respond to particular classes of medications. In pediatrics, a similar corollary is the RISK study cohort[30], the first of its kind with regards to both size and granularity of collected data.

The use of advanced statistical methods will be necessary to take full advantage of these datasets and to explore all potential predictors. Machine learning (ML) and propensity score-based methodologies are two examples that have been increasingly utilized within IBD-related epidemiologic research over the last several years. For example, Waljee and colleagues recently applied random forest modeling to assess predictors of response to vedolizumab in CD and UC within 6 weeks of initiating therapy[196]. This is just one subtype of ML, which includes regression subtypes, spline-based analyses, and neural networking. Utilizing these methods in combination with ensemble machine learning, which can create a weighted model among many of these different ML methods, may help to even further refine these methods as they are applied to new datasets in IBD[197]. Another potential methodology that can be applied with combined large datasets is high-density propensity score matching[198]. Using a large amount of available variables to identify like individuals for comparison allows one to emulate a randomized controlled trial in observational datasets. This was recently demonstrated in a combined cohort of Medicare and Medicaid patients to compare the effectiveness of early anti-TNF use compared to repeated corticosteroid utilization[83]. Propensity-score methods were also employed to compare early anti-TNF therapy to thiopurine monotherapy in the pediatric RISK cohort[143].

Another important challenge that may be amenable to pragmatic clinical research methods is determining how we ideally position different medication classes when considering sequential therapies. As noted, the majority of the patients will not respond or lose response to their initial therapy. Research to date has employed simulation or network meta-analytic methods to attempt to determine ideal sequences of therapies in CD and UC[192, 199-201]. While these studies are an important step, they are also limited for several reasons. Firstly, they combine data from studies with different outcome measures or different follow-up periods. Secondly, these studies often do not incorporate all potential therapy sequences; this is particularly important for infliximab. Thirdly, these studies cannot accurately assess particular patient subgroups: there are limited randomized controlled trial data on many of these agents in pediatric or elderly populations, for example. Lastly, there may be practice heterogeneity in how drugs are used, particularly regarding dose modifications among community providers and those at specialized academic medical centers, or in regions with limited resources.

Obtaining more accurate effectiveness estimates should be a primary short-term goal of clinical studies in IBD over the coming years. Large observational cohorts are well-positioned to assess sequential medication effectiveness as new medications or medication classes come to market, though there may be an initial delay in data acquisition. These data may also potentially be used to expand the knowledge base regarding how to best use these medications in the post-operative setting[202, 203]. Larger datasets also have the potential to help address questions regarding practice or geographic heterogeneity and how that may influence medication effectiveness or sequencing. Recent data suggest geographic variation likely affects IBD outcomes[204]. While larger cohorts will also likely accrue data in subgroup populations, data
collected prospectively by RISK or SPARC IBD will provide much needed granularity to also address how we employ our newest therapies in IBD.

C. Better defining how clinicians are utilizing therapeutic drug monitoring in IBD

There has been growing interest in utilizing therapeutic drug monitoring (TDM) to optimize the effectiveness of immunosuppressive medications in IBD. However, several questions remain. In adult populations, there is an active debate regarding the ideal timing of measuring biologic drug levels and anti-drug antibodies. “Reactive monitoring”, or using TDM when individuals have lost response or have not responded to therapy, has been accepted as an important component of using biologics[205]. The available data assessing “proactive monitoring”, or the use of TDM to assess drug levels in those with an appropriate clinical response, are considerably murkier, however. Two large randomized controlled trials were unable to demonstrate a significant clinical benefit, though critics have highlighted some potential design flaws[206, 207]. Additionally, several observational studies have demonstrated a potential benefit of proactive monitoring for both adalimumab and infliximab[208, 209].

It also remains unclear to what extent TDM is being employed both at and outside of academic medical centers. Identifying inappropriate or inadequate use of reactive TDM would allow organizations such as the Crohn's & Colitis Foundation to provide educational outreach to providers in order to ensure patients have the best chance of response to their therapy. Patterns of test utilization will likely be readily available in claims-based datasets in the next few years. Datasets such as SPARC IBD will also allow us to assess the effectiveness of both reactive and proactive interventions on a larger scale than previously performed. Furthermore, it is unclear what barriers may exist for patients who have been advised to perform therapeutic drug monitoring. Future qualitative research using IBD Partners to determine what insurance or cost related factors may influence testing will be vital. Given likely wide variation in care coupled with the minimal prospective data currently available, this is also an area for potential prospective pragmatic clinical trials assessing different algorithms of reactive and proactive TDM.

Previously published TDM research has been conducted primarily in adults. There remain limited data for pediatric patients, in whom pharmacokinetics may be quite different. Using highly granular data with serum drug levels may better help to determine if target trough ranges are similar in pediatrics, and how TDM may be best utilized in these patients.

D. Studying pain management, opioid use and cannabis

Another important area in IBD care is pain management, and related to this, opioid use. The relationship between opioid use and increased risks of both morbidity and mortality in IBD is well-established[210, 211]. Despite these risks, there are concerning data that the same epidemic trends in opioid use affecting the general population are affecting both adult and pediatric patients with IBD [212, 213], and that prescriptions of opioids to IBD patients during a flare is associated with a higher likelihood of persistent opioid use, as were co-morbid conditions including depression [214]. One potential means for combating opioid misuse
would be the inclusion of pain medicine specialists in IBD care. Addressing co-existent mental health problems and understanding pain medicine utilization patterns, and how they may impact urgent care resource use would allow for future targeted interventions for both patients and providers. Referrals to pain medicine and anesthesia can be readily tracked in claims-based data, and prescribing patterns can be assessed in large secondary datasets with pharmacy files, as well as new datasets such as SPARC IBD.

Finally, another major trend in IBD management is marijuana legalization[215]. Currently, medicinal marijuana (and cannabinoid derivatives) is legal in 9 states with legislature pending in numerous others, and many patients with IBD are already using or inquiring about the use of cannabis and its derivatives. While there are no proven anti-inflammatory effects, marijuana use may impact opioid use: In Colorado, cannabis legalization resulted in a statistically significant reduction of opioid-related overdoses[216]. Marijuana use itself is difficult to track in observational data, though state-specific time-series analyses in relation to time of legalization may help to better assess if there are changes in opioid use over time. More in-depth data will be needed, however, to accurately measure the use and impact of marijuana use over the coming years. Additionally, prospective observational studies such as CANDID at the University of Colorado, are crucial first steps in assessing this. Future prospective data collection using existing patient reported research networks across multiple geographic regions will also be vital. This topic is covered further in a recent Crohn’s and Colitis Foundation white paper on the topic [217].

E. Understanding of health economics and healthcare resources utilization

Over the past 15 years, the utilization of large claims- or electronic medical record-based datasets has allowed us to begin to grasp the economic impact of caring for patients with IBD. In one such study, estimated annual direct charges of IBD exceeded $6.3 billion dollars in 2003[218]. Even after adjustment for inflation, this is likely an underestimate of today’s costs, as biologics have become an ever-growing component of IBD care and related expenditures[219]. These burdens affect pediatric populations as well, with national estimates of $2.9 billion[220]. The out of pocket expenses of these medications can be burdensome to patients and their families[221]. Patients with IBD also often have increased costs associated with co-morbidities, including mental health disorders.

Therefore, there is increased interest in providing comprehensive IBD care, which involves a multi-disciplinary approach, comprised of more than gastroenterology visits and related laboratory studies, imaging, and procedures. For example, we are only beginning to understand the importance of screening for depression, anxiety, and other psychiatric disorders, with growing data suggesting an intrinsic relationship between IBD-related outcomes and depressive symptoms [222]. Despite increasing screening recommendations for depression and anxiety, little is known at this time about geographic or center-based disparities in psychiatric screening, related referrals, or the availability of these resources. Identifying gaps in education or access related to depression and anxiety in IBD would allow us to improve patient care and compliance, potentially reducing hospitalizations and improving quality of life. Using larger data sources
with greater geographic coverage may help to answer these utilization questions, though more granular data may be required to assess the frequency of actual screening practices among gastroenterologists.

**Steps Forward**

From a methodologic standpoint, we are at the fore of an exciting era in IBD research in the US and worldwide. Access to larger patient populations is rapidly expanding, both secondary to the emergence of large claims datasets over the past decade, and the development of new merged databases. Recently developed initiatives such as RISK, fielded through the PRO-KIIDS network, and SPARC IBD, will provide researchers not only with broad patient data across thousands of lives, but do so with a depth and granularity that has not been achieved previously, merging deep clinical phenotyping with laboratory data and samples. IBD researchers have also become versed in the necessary statistical methods to maximally utilize these data, emulating prospective RCTs to predict potential outcomes or adverse events.

It is therefore important, among this rapid proliferation of resources and skills, that potentially attainable clinical goals be highlighted for the research community embarking on pragmatic clinical research over the coming years. Throughout this section, we have highlighted several research topics that are either readily amenable to research in existing datasets, or soon will be as new data are collected. These tools will allow us, in the next several years, to better understand trends in incidence and prevalence of IBD, both in adult and pediatric patient populations. As more patients are exposed to newer agents and their data are accrued in claims and observational data, we will have the ability to better assess patterns and algorithms of care, measuring real world effectiveness in a manner that has not been achievable before. These findings will lead to better drug selection and optimization. Lastly, these data will allow us to best understand how patients are utilizing healthcare resources outside of their gastroenterologists’ office. Understanding psychiatric or pain medicine resource use, how this is changing over time, and how it can impact urgent or emergent care access is vital to providing integrated, comprehensive care for our patients.

To specifically address these research goals over the next several years, the following focuses of pragmatic clinical research should be considered:

1. **Emphasize the use of emerging data sources to better understand epidemiologic and therapeutic trends in IBD, expanding on existing data to better understand how and where we should improve care.**

2. **Better characterize how we can best position medical therapies as new medications and medication classes come to market. These research efforts should include both identifying which therapies are best utilized as first-line treatment, and in whom; as well as those sequences of therapies and timing of therapy that are most effective should treatment failure and/or the need for surgery occur.**

3. **Describe provider and geographic variation in the utilization and effectiveness of both proactive and reactive TDM in adult and pediatric patient populations.**
(4) Identify the impact of ethnic diversity and geographic variation in IBD care in the US, including medication treatment patterns, access to support services, psychiatric utilization and comprehensive IBD center of excellence referrals and use. An additional goal would be to determine how access to these resources influences medication persistence and clinically relevant outcomes in IBD.

(5) Support and promote future prospective pragmatic studies with clustered randomization to assess:

   i) positioning of therapeutic options, especially new classes of agents and timing around surgery
   ii) TDM algorithms and their impact on durability of response, cost, HC utilization
   iii) Impact of multi-disciplinary psychiatric care/pain support services on outcomes such as ER and urgent care utilization, pain management, medication compliance, rates of hospitalization, and medication persistence.

**REFERENCES**


84. Hoarau, G., et al., *Bacteriome and Mycobiome Interactions Underscore Microbial Dysbiosis in Familial Crohn’s Disease.* MBio, 2016. 7(5).


204. Borren, N.Z., et al., Distance to Specialist Care and Disease Outcomes in Inflammatory Bowel Disease. Inflamm Bowel Dis, 2017. 23(7): p. 1234-1239.


