PROTOCOL

Predicting Response to Standardized Pediatric Colitis Therapy (PROTECT)

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1. SUMMARY

This multicenter open-label study is designed to evaluate the safety and efficacy of standardized initial therapy using either mesalamine or corticosteroids then mesalamine for the treatment of children and adolescents newly diagnosed with ulcerative colitis.

The study will investigate the hypothesis that response to the initial 4 weeks of therapy as well as specific clinical, genetic, and immune parameters determined during the initial course of therapy will predict severe disease as reflected by need for escalation of medical therapy or surgery.

A total of up to 475 subjects will be assigned to one of two initial therapeutic plans (mesalamine only or prednisone/liquid equivalent prednisolone followed by mesalamine) depending upon initial disease severity determined by the validated multi-dimensional Pediatric Ulcerative Colitis Activity Index (PUCAI). Biospecimens (blood, stool, colonic tissue) will be obtained at diagnosis, and subsequently following the initiation of therapy at weeks 4, 12, and 52 (blood and stool at weeks 4 and 12; blood, stool, and colonic tissue at week 52). Clinical evaluation will take place at pre-specified time points over up to two to five years of follow up. Adherence to mesalamine dosing will be monitored using a state of the art electronic Medication Event Monitoring System (MEMS®).

The primary endpoint is corticosteroid free remission (SFR) at 52 weeks on mesalamine therapy only without the need for rescue therapy with immunomodulators (IM), calcineurin inhibitors (CI), anti-TNFα therapy, or surgery. Secondary endpoints include steroid free remission (SFR) at other time points, PUCAI<10 at 4 weeks, colectomy during follow-up, quality of life measures and, in a subset of patients, endoscopic remission or response at 52 weeks.

2. STUDY DEFINITIONS

2.1. Schroeder Endoscopic Scoring System (Mayo Score)

Endoscopic severity will be assessed using the Mayo sigmoidoscopy score [1]:

0 = Normal or inactive disease
1 = Mild disease (erythema, decreased vascular pattern, mild friability)
2 = Moderate disease (marked erythema, absent vascular pattern, friability, erosions)
3 = Severe disease (spontaneous bleeding, ulceration)

2.2. Pediatric Ulcerative Colitis Activity Index (PUCAI)

The PUCAI is scored using the following table, based on information obtained by a health professional who has evaluated the subject [2]:

ITEM	POINTS
1. Abdominal pain:	
No pain Pain can be ignored Pain cannot be ignored	0 5 10
2. Rectal Bleeding	
None Small amount only in less than 50% of stools Small amount with most stools Large amount (>50% of the stool content)	0 10 20 30
3. Stool consistency of most stools	
Formed Partially formed Completely unformed	0 5 10
4. Number of stools per 24 hours	
0-2 3-5 6-8 >8	0 5 10 15
5. Nocturnal stools (any episode causing awakening)	
No Yes	0 10
6. Activity level	
No limitation of activity Occasional limitation of activity Severe restricted activity	0 5 10
SUM OF PUCAI (0-85)	**0-85**

The range of possible scores is 0-85 and the classification within this range is:

PUCAI<10	Inactive disease
PUCAI 10-34	Mild activity
PUCAI 35-64	Moderate activity
PUCAI ≥65	Severe activity

2.3. Corticosteroid (CS) Status

CS-free: Subjects will be considered to be CS-free if they are taking no CS at the assessment and have been CS-free for a minimum of 2 weeks at that point. Alternate day CS will not be considered CS-free.

CS responsive: Clinical improvement and termination of CS within 12 weeks of initiating therapy, and remaining off CS for the remainder of the first year or a minimum of six months following initial tapering before requiring re-treatment with CS.

CS dependent: Required CS >12 weeks to control symptoms, or at least one additional course of CS starting <6 months after initial successful CS weaning

CS refractory: Unresponsive to oral CS within four weeks of starting therapy and required rescue therapy with CI or anti-TNFα therapy; or required rescue therapy with CI or anti-TNFα therapy because of failure to respond to intravenous CS, or required colectomy because of failure to respond to CS.

2.4. Rescue therapy

The use of immunomodulators (IM), calcineurin-inhibitors cyclosporine or tacrolimus (CI), anti-TNFα inhibitors, or colectomy in patients with ulcerative colitis is referred to as rescue therapy.

2.5. Abbreviations and Acronyms

5-ASA	5-aminosalicylic acid (mesalamine)
AUC	area under the (receiver operating characteristic) curve
BASC-2	Behavior Assessment System for Children – Second Edition
CD	Crohn's disease
CI	calcineurin inhibitors
CS	corticosteroid
CRF	case report form
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
GR	glucocorticoid receptor
IBD	inflammatory bowel disease
IM	immunomodulators
IRB	Institutional Review Board
MEMS®	Medication Event Monitoring System
MH	mucosal healing
NPV	negative predictive value
OPG	osteoprotegerin
OR	odds ratio
PPD	purified protein derivative
PPV	positive predictive value
PRO-KIIDS	Pediatric Resource Organization for Kids with Intestinal Inflammatory Disorders
PUCAI	Pediatric Ulcerative Colitis Activity Index
RDCRC	Rare Disease Clinical Research Consortium
SAE	serious adverse event
SFR	corticosteroid free remission
TPMT	thiopurine methyltransferase
UC	ulcerative colitis

3. BACKGROUND AND SIGNIFICANCE

3.1. Overview

Ulcerative Colitis (UC) denotes a phenotype of chronic inflammatory bowel disease (IBD), where inflammation is localized to the colonic mucosa, and extends from the rectum proximally in varying extents. The disorder is thought to result from an inappropriate activation of the mucosal immune system by antigens derived from both the host epithelium and the enteric flora, in genetically susceptible individuals. UC is strikingly heterogeneous with respect to age of onset, anatomical extent and disease course, with some patients experiencing chronically active severe disease, while others have intermittent periods of clinical remission and disease exacerbation. Patients' therapeutic responses are also equally variable. The reasons underlying such variability are not well understood. Although it has been widely hypothesized that several genes may influence the development of UC, and modify its phenotypic expression and severity, to date there are few confirmed examples of such relationships. Recently, several investigators have completed a genome-wide association study (GWAS) in which variation in *TNFRSF6B* was linked to risk for UC in children [3]. The *TNFRSF6B* gene product, decoy receptor 3 (DCR3), regulates monocyte and lymphocyte function, and prolongs lymphocyte survival by inhibiting Fas signaling [4]. As chronic lymphocyte activation and resistance to activation-induced cell death are felt to be fundamental mechanisms of mucosal inflammation in UC, it is likely that variation in *TNFRSF6B* regulates both susceptibility for UC, and behavior of established disease [5].

3.2. Comparing Pediatric Onset to Adult Onset Ulcerative Colitis

In comparison with adult-onset disease, it is consistently observed that UC developing during childhood is more likely to be severe and extensive [6, 7]. Given the higher proportion of extensive disease seen in children, a more frequent occurrence of severe/fulminant UC might also be anticipated. Indeed, while adults with UC are reported to have a 15% lifetime risk of experiencing a severe exacerbation, which prior to corticosteroid (CS) therapy was associated with significant mortality, in a recent retrospective analysis of children with UC residing in the Greater Toronto Area we calculated a 28% rate of hospitalization due to severe exacerbation over a median follow-up of six years [8]. CS failure rate (46%) was also higher among these hospitalized children (n=100), than had been observed in a previous systematic review of published cohort studies of hospitalized adults (29%)[9]. Two of the three other previously reported, but very small (total n=43), studies evaluating outcome of admissions for severe pediatric UC mirrored the Toronto Hospital for Sick Children experience [10-12]. Interestingly, among the 100 children hospitalized in Toronto, the colectomy rate after one-year remained remarkably stable, confirming data suggesting that most UC-related admissions occur during the first few years following diagnosis.

3.3. Current Management of Pediatric Ulcerative Colitis

Basic induction therapy usually includes aminosalicylates in subjects with mild to moderate disease, and aminosalicylates and CS in those with moderate to severe disease; inadequate response often prompts the use of immunomodulators (IM), calcineurin inhibitors (CI; cyclosporine, tacrolimus), or infliximab.

3.3.1. Aminosalicylates

It has been postulated that the 5-aminosalicylate drugs exert their anti-inflammatory effect locally at the intestinal mucosa [13, 14]. Mechanisms likely include inhibition of 5-lipoxygenase with resulting decreased production of leukotriene B4, scavenging of reactive oxygen metabolites, prevention of up-regulation of leukocyte adhesion molecules, and inhibition of IL-1 synthesis. Because 5-ASA is rapidly absorbed from the upper gastrointestinal tract on oral ingestion, different delivery systems have been used to prevent absorption until the active drug can be delivered to the distal small bowel and colon. Pentasa® (mesalamine) the 5-ASA preparation to be used in this study coats microgranules of mesalamine with ethylcellulose, releasing it in a time-dependent fashion.

Though multiple studies have shown the efficacy of aminosalicylates in inducing and maintaining remission in adults with ulcerative colitis [15-17], there are few data in children. Indeed, with the exception of one small study comparing olsalazine to sulfasalazine in mild to moderate UC [18], there have been no controlled studies. Other reports have all been single center anecdotal experiences with small numbers of patients [19, 20]. The ideal dose of 5-ASA in the treatment of pediatric ulcerative colitis is also uncertain with doses from 30-80 mg/kg/day being noted [20]. In adults it has been suggested that higher doses are more effective than lower doses [21, 22].

Adverse reactions to all of the 5-ASA preparations have been described, requiring discontinuation in 5-15% of cases. More common side-effects include headache and abdominal pain, which often improve with time and infrequently require cessation of therapy. Approximately 5-10% of treated cases of ulcerative colitis develop paradoxical worsening of diarrhea and/or bleeding on 5-ASA requiring that the medication be stopped. Pancreatitis is seen in up to 3% of treated cases and does not appear to be dose-dependent; it usually occurs within the first month or two of starting therapy. Nephritis is rare but periodic urinalysis and measurement of renal function are recommended.

3.3.2. Corticosteroids

Corticosteroids have constituted the mainstay of treatment of severe UC since efficacy was first demonstrated in the 1955 randomized controlled trial by Truelove and Witts [22]. Recent practice guidelines developed in adults support their use because of rapid onset of action and significant efficacy [23] though CS dependency is noted [24]. Though no controlled data on their use have been reported in children they are frequently used in this population [25]. A recent report from investigators leading the prospective Pediatric IBD Collaborative Research Group Registry [26] described 97 subjects with a diagnosis of UC and a minimum of 1 year follow-up; 77 (79%) received CS (62 within 30 days of diagnosis (early) and 15 between 31 days and 6 months (late)). At diagnosis 81% of CS treated patients (age 11.3 ± 3.5 years) had moderate/severe disease, and 81% had pancolitis. Clinically inactive disease as determined by physician global assessment was noted in 60% at 3 months following CS therapy, but by one year 45% were considered CS dependent despite the frequent use of IM. Among those children with initially moderate to severe disease in clinical remission at 3 months, about two-thirds had stopped the CS by one year. In this patient cohort there were no standardized CS dosing regimens or tapering schedules.

3.3.3. Adherence and Clinical Outcomes

Treatment regimens for pediatric UC involve many tablets at different times of the day and can have unpleasant side effects. Our research has identified non-adherence prevalence rates ranging from 50-88% across medications in pediatric IBD [36-38]. These data are alarming given that the risk of relapse in UC is 5.5 times greater in non-adherent patients than in adherent patients, and the annual costs of health care for non-adherent UC patients are 12.5% higher than for adherent patients [39]. Work from our group has shown that internalizing problems such as depression are often associated with non-adherence in pediatric IBD patients; other barriers to adherence include forgetting, being away from home, and interference with an activity [40]. Data from an ongoing study of self-management behavior in pediatric IBD in a sample of 62 adolescents, led by our co-investigator Dr. Hommel, has confirmed that reduced medication adherence is associated with increased disease severity after statistically controlling for the effects of age, sex, disease sub-type, and internalizing behavioral symptoms (e.g., depression) [41]. However, no study has prospectively defined rates or consequences of non-adherence to aminosalicylates in pediatric UC.

3.4. Genetic Modifiers and Genomic Predictors

3.4.1. Genetic Modifiers of Host Response and Potential Effect on Disease Course

Although the major goal of genetic research has been to elucidate etiopathogenesis, it is hypothesized that an understanding of genetic variation may also help explain much of the tremendous heterogeneity that has been observed in phenotypic expression, natural history, and response to therapy. While the molecular determinants of UC progression and treatment response remain to be fully elucidated, it seems reasonable to assume that at least some of the factors and processes that originally modulate disease initiation will also be at play in disease progression. For instance, the most consistently replicated HLA associations with UC are *DRB1*0103* and *DRB1*15* [42-46]. The association with both of these variants is particularly strong in patients with extensive or severe disease. A biallelic structural polymorphism in the inhibitor of κB-like (*IKBL*) gene, in the central MHC region has also been associated with severe UC [45]. While some of the polymorphisms of the cellular export pump *MDR1* have been generally associated with UC susceptibility, other polymorphisms (C3435T) have been specifically associated with CS treatment response [47, 48]. This polymorphism is associated with increased intestinal MDR1 expression, and presumably altered cellular CS metabolism. Recently, results of a nonsynonymous SNP scan for ulcerative colitis identified a previously unknown susceptibility locus at *ECM1* [49]. It was also shown that several risk loci are common to UC and Crohn's disease (*IL23R, IL12B, HLA, NKX2-3 and MST1*). A recent German study identified *3p21.31*, *NKX2-3*, and *CCNY* as susceptibility factors for both Crohn's disease and UC, and variants in *PTPN2*, *HERC2*, and *STAT3* associated with UC only [50]. The recent meta-analysis which included both adult- and pediatric-onset cases increased the number of UC risk loci to 47, and implicated biologic pathways involved in barrier function, autophagy, and adaptive immune responses [31].

Most relevant to the current study, a recent report examined Genome Wide Association Study (GWAS) data from 324 adult UC patients who required colectomy for refractory disease compared to 537 adult UC patients who did not require colectomy [51]. Colectomy was associated with pancolitis and a positive family history of UC, two factors which are more common in pediatric-onset disease. Colectomy was not associated with age at onset or gender.

A risk score based on the combination of 46 single nucleotide polymorphisms (SNPs) associated with refractory UC accounted for 48% of the variance for colectomy risk in this cohort. Risk scores divided into quartiles showed the risk of colectomy to be 0%, 17%, 74%, and 100% in the four groups. The area under the curve (AUC) for the risk score was equal to 0.91, with a sensitivity and specificity over 90%. Whether the genetic variants identified in this cohort or the recent GWAS meta-analysis may also influence disease behavior including the response to therapy in pediatric UC is not known.

3.4.2. Genomic Predictors of Response to Therapy and Disease Course.

Several studies from our group and others have examined the global pattern of gene expression in the colon of UC patients at diagnosis and during therapy. We found that colonic STAT3 activation and biologic networks driving leukocyte recruitment, HLA expression, angiogenesis, and tissue remodeling are enriched in the colon of children with UC at diagnosis [52]. Subsequently, genetic variation in *STAT3* was found to control risk for the development of IBD. Investigators from our group have utilized microarray from RNA isolated from peripheral blood mononuclear cells (PBMC) of children with UC receiving intravenous corticosteroids to identify a panel of 10 genes which predicted clinical response with over 80% sensitivity and specificity [53]. These included *ABC4*, *MMP8*, and *CEACAM1*. More recently, microarray has been used to identify colonic genes which may predict the response to inflixmab in adults with UC [54]. A panel of five genes (osteoprotegerin (*OPG*), stanniocalcin-1, prostaglandin-endoperoxide synthase 2 (*COX2*), interleukin 13 receptor alpha 2 and interleukin 11) separated responders from non-responders with 95% sensitivity and 85% specificity. Remarkably, our group has now reported that elevated fecal OPG is associated with lack of response to intravenous corticosteroids in pediatric UC [55]. Whether determination of the global pattern of colon gene expression by RNA-Seq or microarray may be used to identify a panel of colonic genes at diagnosis of pediatric UC which will predict clinic outcomes is not known.

3.5. Environmental Factors

3.5.1. Epidemiology and Environmental Factors

The incidence of both CD and UC has continued to increase during the past decade, particularly in pediatric populations reported from both North America and Europe [56]. In a retrospective study of pediatric IBD in Northern California from 1996-2006, the incidence of UC increased significantly by 2.7 fold and CD increased two fold [57]. Similarly, an epidemiologic review of pediatric IBD globally revealed increased incidence of both UC and CD [58]. Specifically, IBD appears to be increasing in parts of the world including Asia and South America where it was previously uncommon [59].

This heightens the importance of ongoing studies to define environmental triggers for disease development. Studies in Scotland have implicated higher socioeconomic status (without change in genetic susceptibility) as an important factor in the development of IBD [60]. This suggests early life environmental exposures may potentially be associated with IBD. Microbial exposures in childhood related to the “hygiene hypothesis” have been explored including H pylori, helminthes, and other childhood infections. Other factors modifying the response to infection including childhood vaccinations, antibiotic exposure, mode of delivery and breastfeeding have also been considered. However, currently only two environmental

influences, smoking and appendectomy, are well established risk factors for IBD [61, 62]. While a detailed consideration of environmental factors which may contribute to the pathogenesis of pediatric UC is beyond the scope of PROTECT, we will consider the role of vitamin D deficiency in Aim 2.

3.5.2. Vitamin D and Immune Regulation

Recent registry studies have demonstrated a high rate of vitamin D deficiency in children with CD and UC [27], and vitamin D regulates innate and adaptive components of the mucosal immune response which could contribute to IBD clinical outcomes. For example, the expression of cathelicidin and defensins is under the control of vitamin D. These natural antibiotics are part of the innate immune system that controls the microbial populations that colonize intestinal mucosal surfaces [27, 28]. Patients with UC have elevated β-defensin-2 and cathelicidin in the colon, a protective response that may be blunted in vitamin D deficiency [29]. Vitamin D induces autophagy, a process that recycles defective intracellular organelles and plays a role in bacterial killing. Loss-of-function polymorphisms in autophagy *ATG16L1* and *IRGM1* genes are susceptibility factors for CD and defects in autophagy secondary to vitamin D deficiency may worsen the course of UC [30]. Importantly, a recent meta-analysis has identified *DAP* (death-associated protein), a negative regulator of autophagy, as a susceptibility gene for UC [31]. Vitamin D also regulates T cell activation and the phenotype and function of antigen-presenting cells (APC), particularly dendritic cells (DCs) [32]. In a rodent model of colitis, DCs preferentially polarize naïve T cells toward a regulatory phenotype under the influence of vitamin D [32]. Consistent with this, resistance to vitamin D results in fulminant disease in murine colitis [33]. Supplementation with $1,25(OH)_2D$ reduced both serum CRP and clinical disease activity by week 6 in adult CD patients [34]. In a recent randomized controlled trial, oral vitamin D supplementation reduced the one year rate of relapse in adult CD patients from 29% to 13% [35]. It is not known if vitamin D deficiency is associated with dysregulated immune responses and worse clinical outcomes in children with UC.

3.5.3. Enteric Flora

Studies using newer molecular methods have begun to characterize fundamental differences in the enteric flora between children with IBD and healthy controls [63]. The microbiota of the ileum and colon contains a variety of metabolically active bacteria that interact with the host mucosal immune system. Inappropriate activation by the intestinal microbiota is thought to play an important role in the etiology of IBD [64]. A breakdown in the balance between protective and harmful bacteria (dysbiosis) is a current prevailing hypothesis for the development of disease [65]. Bacterial load and the nature of the commensal flora can influence both the site and degree of GI inflammation [66-68]. Some changes in the intestinal microbial community are shared in UC and CD including increased concentrations of *E. coli* [69, 70], presence of non-commensals [65, 71, 72] and reduced diversity [71]. Bacterial populations have also been found to vary based on CD phenotype. Patients with ileal disease may have disappearance of *Faecalibacterium* and *Roseburia* with increased numbers of *Enterobacteriacea* and *Ruminococcus gnavus* [73]. The role the microbiota plays in the pathogenesis of IBD will continue to be a very active area of investigation. Results of a large prospective cohort study of high-risk families, and of the composition of the enteric flora in twins divergent for IBD, are eagerly awaited. While a detailed characterization of the enteric

flora would be beyond the scope of PROTECT, we will store mucosal DNA and fecal samples which may be used in future ancillary microbiome studies. These will likely be conducted using new molecular and bioinformatics approaches which are being developed by the recently initiated CCFA Microbiome Consortium.

3.6. Serological Responses and Potential Relationship with Disease Course

The relationship between serological response to microbial and auto-antigens and disease course in UC remains largely unexplored. In principle, serological status may be an indicator of ongoing antigen exposure due to disrupted mucosal integrity and, as such, may be predictive of more persistent disease that will require an escalation in therapy. In CD, ASCA, antibodies to *Escherichia coli* outer membrane porin C (anti-OmpC) and antibodies to CBir1 flagellin (anti-CBir1) have been shown to predict disease progression [74]. One study in UC suggested that pANCA (perinuclear anti-neutrophil cytoplasmic antibody) positivity was associated with increased resistance to treatment in left-sided colitis [75]; another has shown that the response to infliximab is better in adult UC patients who are pANCA sero-negative [76]. The presence of OmpC and pANCA seem to be predictive of pouchitis following ileoanal pouch procedure [77]. Immunization of mice to flagellin has been shown to exacerbate colitis due to dextran sodium sulfate exposure, suggesting that these antibodies may play a role in disease pathogenesis [78]. Recently, we have reported for the first time that high titers of circulating Granulocyte Macrophage Colony Factor (GM-CSF) auto-antibodies (Ab) are associated with refractory disease requiring surgery in pediatric and adult CD [79]. We found that GM-CSF was required for gut barrier function in both animals and pediatric CD patients, and that loss of GM-CSF bioactivity exacerbated experimental gut injury [80]. In our recent preliminary studies we have determined that GM-CSF Ab are produced by lamina propria cells (LPMC) isolated from the inflamed colon in UC, and that adult UC patients with higher circulating levels of GM-CSF Ab are more likely to require colectomy. Whether neutralizing GM-CSF Ab are also associated with disease outcomes in pediatric UC is not known. We will examine the relationship between these serologic markers and longitudinal disease course in UC.

3.7. Ulcerative Colitis and the Inflammatory Response

In the normal host, inflammation is generally a two-phase process. Cytokines such IL-6, IL-1β and TNF-α are released in response to an initial inflammatory trigger and activate a series of pro-inflammatory transcription factors including nuclear factor-κB (NF-κB) and activator protein 1 (AP-1). Consistent with this, we have recently identified IL-6:STAT3 dependent biologic networks up-regulated in the colon of children with UC at diagnosis which drive leukocyte recruitment and survival [52]. These in turn lead to the production of various pro-inflammatory chemokines and cytokines including IL-8, IL-12, IL-15, IL-17 and IL-18 [81]. In balance, anti-inflammatory agents including cytokines, such as INFα and IL-10, as well as cortisol, secreted in response to ACTH from the pituitary, are also released [82]. In patients with UC, a variety of cytokine abnormalities have been reported, even during periods of clinical remission. For instance, the over-expression of IL-1β and IL-8 in colonic mucosa has been repeatedly demonstrated [83-91]. Similar observations for the inflammatory cytokines IL-6 and TNFα have also been reported, but less consistently[84, 87, 88, 90-92]. Interestingly, elevation of the anti-inflammatory cytokine IL-10 within the inflamed colonic mucosa of patients with UC has also been demonstrated, although, again, not universally [83, 89, 91, 93]. To date, however, alterations in IL-18 and IL-12 have not been reported in UC [94]. Lamina

propria T cells from UC patients produce significantly greater amounts of IL-5 and IL-13 than control cells and little IFN-gamma, whereas comparable cells from CD patients produce large amounts of IFN-gamma and small amounts of IL-13 [95]. Stimulation of UC lamina propria T cells bearing an NK marker (CD161) with anti-CD2/anti-CD28 or with B cells expressing transfected CD1d induces significant IL-13 production. These studies suggest that UC is associated with an atypical Th2 response mediated by non-classical NKT cells producing IL-13 and having cytotoxic potential for epithelial cells [95]. Another critical feature is the resistance of the mucosal T lymphocytes to activation-induced cell death, which has been linked to increased production of decoy receptors including DCR3 [5]. Collectively, these studies are quite consistent with our discovery of *TNFRSF6B*, the gene which encodes DCR3, as a susceptibility gene for pediatric UC [3]. However, whether a specific mucosal cytokine profile is associated with clinical outcomes in pediatric UC is not known.

3.8. Characterizing Patients by Corticosteroid Response

CS have been the mainstay of therapy for moderate or severe UC; disease course is often characterized in terms of being CS-responsive, CS-dependent, or CS-refractory.

3.8.1. Corticosteroid Pathway and its Underlying Molecular Mechanism

CS readily cross the cell membrane and bind to the cytosolic glucocorticoid receptor alpha (GRα). In the absence of CS, the inactivated GRα is bound to a multi-protein structure within the cytoplasm which includes, among others, the proteins HSP90 and calreticulin [82, 96, 97] . In the presence of CS, GRα dissociates from this multi-protein structure, and the joint CS/GRα complex migrates to the nucleus and binds to the glucocorticoid responsive elements (GRE). Within the nucleus, the CS/GRα complex mediates various positive and negative reactions. Through an interaction with transcription factors such as AP-1 and NF-κB, the complex inhibits the transcription of pro-inflammatory factors [81, 98], thus decreasing systemic inflammation.

Some of the variability in CS-responsiveness may stem from differences in disease severity; but underlying heterogeneity in molecular pathways involved in the CS response may also be important. It has been demonstrated in clinical studies that CS therapy failure is more common in patients with severely active disease [8]. Furthermore, there have been *in vitro* studies of T-cells isolated from UC patients who had failed to respond to CS therapy, whereby the cells obtained during the acute flare demonstrated CS resistance, while those obtained 3 months later, following colectomy, did not [99]. In contrast, there have been numerous clinical studies in both IBD and rheumatoid arthritis which have demonstrated ***no*** correlation between disease activity and *in vitro* CS response [82, 100]; whereas there have been various *in vitro* studies on samples from patients with a variety of inflammatory diseases where abnormal T-cell proliferation patterns ***have*** persisted during periods of inactive disease [101-103]. Even lymphocytes collected from healthy volunteers have a heterogeneous *in vitro* response to CS stimulation. Taken together, these observations suggest that it is a combination of factors, relating to both intrinsic individual mechanisms and to underlying disease activity, which account for CS resistance in UC. Any investigation examining predictors of CS response must consider both.

3.8.2. Putative Explanations for Variation in Corticosteroid Response

Variation in CS response has been related to both underlying genetic variation, as well as specific cytokine profiles.

The Glucocorticoid Receptor (GR): The GR (encoded by *NR3C1* on chromosome 5 at 5q31) has various isoforms (α, β, γ, P, A); however, only GRα is pharmacologically functional [82, 96]. Although produced in much smaller quantities, GRβ has a much longer half-life than GRα. Interestingly, it has been demonstrated to inhibit GRα [104, 105]. While its physiologic role remains uncertain, increased GRβ expression has been associated with CS resistance in both asthma and UC patients [106-109]. To date, various genetic polymorphisms within the GR gene have been shown to differentiate CS responsive from resistant patients [96]. While some variants have been associated with CS hypersensitivity, others have been associated with a reduced CS response. In some instances, this has been related to increased GRβ stability, but in most instances the mechanism of associated CS resistance has not been ascertained [96, 110-117].

Macrophage migration inhibitory factor (MIF): MIF is excreted from the pituitary gland and various immune cells including macrophages and dendritic cells. It induces the secretion of various pro-inflammatory cytokines including TNFα, IL-6 and IL-1B. It also regulates IL-2 secretion as well as T-cell proliferation [118, 119]. As such, the induction of MIF can effectively counteract the immunosuppressive effects of CS. Indeed, the importance of this molecule in the development of colitis per se has been clearly demonstrated in a murine model [120]. An association with a single polymorphism within the gene for this molecule (*MIF*-173G>C) and CS resistance has been variably reported in children with juvenile arthritis [121-124].

Cytokine stimulation and up-regulation: Various studies have demonstrated the tight interplay between the levels of specific cytokines and CS resistance. For instance, the joint effect of IL-2 and IL-4 is to induce the phosphorylation of GR by p38MAPK, thereby reducing its affinity for CS, and increasing the levels of GRβ [124, 125]. Indeed, even when healthy donor lymphocytes are incubated with IL-2 and IL-4, the expression of GRβ increases and steroid resistance is induced [108]. Similarly, TNFα, IL-8 and IL-1β have also all been shown *in vitro* to induce GRβ expression (and thus steroid resistance) [126, 127] with clinical trials demonstrating higher colonic levels of these cytokines in UC patients refractory to CS compared to those responsive to such therapy [87]. Of note, IL-10 acts in an opposite manner and increases CS sensitivity [126, 127]. A sub-group of asthmatic patients resistant to CS therapy have been recognized as having a defect in IL-10 secretion [128]. In fact, carriage of the -1082 AA *IL-10* genotype (low producer) may be a relevant risk factor for developing steroid-dependent IBD [129].

Given the above observations, temporary CS resistance mediated by pro-inflammatory cytokines, or defective up regulation of IL-10, may well occur, leading in turn to an observation of steroid resistant disease.

3.9. Assessing Success and Monitoring Disease Activity in Ulcerative Colitis

Disease course is generally described in terms of a patient's response to therapy: the ability to induce remission, the frequency of relapse and the dependence on CS. Escalation in therapy is usually due to an inadequate response to previous therapeutic interventions. Thus, the 'natural

history' of UC is intimately entwined with therapeutic exposure. Ideally, any study aimed at predicting outcome must recognize this and endeavor to incorporate both features into its design.

In order to study the longitudinal disease course of a chronic relapsing and remitting disease, a reliable method of assessing disease activity is required. The Pediatric Ulcerative Colitis Activity Index (PUCAI) has been developed by and validated for use as a non-invasive measure of disease activity in pediatric UC [2]. The PUCAI scores 6 areas (abdominal pain, degree of rectal bleeding, stool consistency, number of stools per 24 hours, nocturnal stooling, and activity level) with a minimum and maximum score of zero and 85, respectively. It has been shown to have an excellent correlation with physician global assessment, colonoscopic appearance, and Mayo endoscopic severity score [2].

The relevance of specifically ascertaining mucosal healing (MH) in addition to clinical response, and its relationship to 'disease activity' is being increasingly studied [130-133]. As confirmed in a recent meta-analysis [134], clinical trials in UC that use endoscopy as an endpoint have a significantly lower placebo rate, highlighting the degree of discord that exists between disease activity indices and the condition of a patient's mucosa. Current data, mostly gained from adult trials, indicate that MH, particularly with CS, is more often achieved in UC than in CD. What is less certain, however, is whether early MH improves long-term disease outcomes in UC. Certainly, observations in CD (where MH has been associated with longer periods of clinical remission, fewer hospitalizations and fewer surgical interventions [131, 135] support the longer term utility of achieving MH. Minimal similar data, however, are available for UC, although a recent cohort study of adult patients from Norway did observe a notably lower rate of colectomy within 5 years for patients with early MH compared to those without [136]. This was recently confirmed by a prospective cohort study from Italy in which mucosal healing at week 12 following the initial course of CS for adult UC was associated with significantly less requirement for immunosuppressive therapy or colectomy during follow-up[137]. No similar pediatric data exist.

Unfortunately, routine endoscopic re-assessment to ascertain MH is more difficult in children than in adults given the need for sedation/anesthesia in the former group. Thus, if achieving early MH does, indeed, prove an important determinant of longer-term UC outcomes, identifying an effective non-invasive marker of MH will be paramount. To date, there remains no single, universally accepted, non-invasive *biological* marker of disease activity in Pediatric IBD. While patients with active IBD can have altered levels of acute phase proteins, elevated ESR and thrombocytosis [138, 139], these markers are not always reliable in children and adolescents [138]. Members of the S100 family (a group of binding proteins specifically expressed by granulocytes) may potentially provide better biological evidence of gut inflammation [140]. One of the proteins from this family is calprotectin. Calprotectin is a calcium binding protein found within the neutrophil cytosol that is released with cell death or activation, and has therefore been studied as a marker of intestinal inflammation. It is stable at room temperature for one week, and up to one year if frozen. It can be measured from a random, spot stool using a commercially available ELISA which has excellent inter- and intra-observer reproducibility. Fecal levels of calprotectin have been shown to be elevated in active disease, to fall with treatment, and to correlate very well with endoscopic findings [140, 141]. Furthermore, a cutoff of 150 mcg/gm in patients with quiescent IBD is a highly sensitive (90%) and specific (83%) predictor of disease relapse within 12 months [142]. Minimal data

are available from pediatric clinical trials utilizing biological and formal disease activity indices concurrently.

3.10. Need for a well-designed study

Multi-center registry data concerning incident cases of pediatric UC have served to characterize the spectrum of illness at presentation. There are no studies in which drug treatment has been standardized according to a set protocol, nor has there been an attempt to better characterize the inflammatory process and to consider how serologic or genetic factors influence the presentation and severity of disease. In no outpatient study to date have biological and molecular data been prospectively collected in parallel with thorough clinical data. The DNA collected in PROTECT will be available for assessment of UC susceptibility genes, currently and yet to be identified, and, equally importantly, for assessment of polymorphisms in genes associated with CS and aminosalicylate response. The value of serologic and fecal inflammatory markers in predicting disease course as well as relapse off CS in those subjects who previously responded to CS will be assessed.

Overall, this multi-center cohort of pediatric UC will facilitate assessment of outcomes with best current treatments, will allow study of factors important to pathogenesis, and will form the basis of ongoing modifications of therapeutic regimens based on clinical, genetic and serologic variables. As such, it will represent the first large-scale attempt to advance knowledge and improve outcomes in this rare and often devastating pediatric disease. Importantly, members of the PROTECT investigators group have been actively involved in developing a North American pediatric IBD Quality Improvement (QI) network over the past several years, ImproveCareNow (ICN) [143]. ICN members care for over 10,000 children at more than 30 pediatric hospitals in the United States and Canada. This has provided a framework for translation of clinical trials of 6-mercaptopurine and infliximab in pediatric CD to practice, with substantial improvements in remission rates across the collaborative. It is anticipated that ICN will provide the infra-structure to rapidly spread findings from PROTECT to the larger pediatric IBD community.

4. STUDY HYPOTHESES

We hypothesize that in children presenting with ulcerative colitis initial response to the initial 4 weeks of therapy as well as specific clinical, genetic, and immune parameters determined during the initial course of therapy will predict subsequently severe disease as reflected by need for escalation of medical therapy or surgery.

4.1. Primary Hypothesis

Hypothesis 1. Relative to other patients, those who have a PUCAI<10 at 4 weeks from start of therapy will be more likely to be in SFR (PUCAI<10) at 52 weeks while receiving the aminosalicylate mesalamine only as maintenance therapy without the need for rescue therapy with IM, CI, anti-TNFα, or surgery.

4.2. Secondary Hypotheses

Hypothesis 2. Relative to other patients, those who have a PUCAI<10 at 4 weeks from start of therapy will be more likely to be in corticosteroid free remission (PUCAI<10) at 12, 26 and 104 weeks while receiving mesalamine only as maintenance therapy without the need for rescue therapy with IM, CI, anti-TNFα, or surgery.

Hypothesis 3. Relative to other patients, those who have a PUCAI<10 at 4 weeks from start of therapy will have lower colectomy rates through 104 weeks.

Hypothesis 4. Relative to other patients, those who have a PUCAI<10 at 4 weeks from start of therapy will be more likely to have Mayo endoscopy sub-score of 0 or 1 at 52 weeks.

Hypothesis 5. A prediction model based on factors supported by preliminary data and measured at weeks 0, 4, and/or 12 will have good sensitivity and specificity (each ≥ 0.75) for the 52-week outcome of SFR.

Hypothesis 6. Relative to patients who have poor mesalamine adherence (< 80% of prescribed medication taken), those with good adherence (≥ 80% of prescribed medication taken) will be more likely to be in SFR (PUCAI<10) at 52 weeks while receiving mesalamine only as maintenance therapy without the need for rescue therapy with IM, CI, anti-TNFα, or surgery

Hypothesis 7. Relative to patients who are not in SFR at 52 , those who are in SFR at 52 weeks are less likely to require colectomy by terminal follow up visit (2 to 5 years from diagnosis).

Hypothesis 8. Relative to patients who do not achieve a Mayo endoscopy sub-score of 0 or 1 at week 52, those who achieve a Mayo endoscopy sub-score of 0 or 1 at week 52 are less likely to require colectomy by terminal follow up visit (2 years (104 weeks) to 5 years from diagnosis).

Hypothesis 9. Relative to other patients, those who achieve SFR on mesalamine only without the need for rescue therapy at 52 weeks are less likely to require the addition of immunomodulators or anti-TNFα therapy by week 104.

Hypothesis 10. A panel of genes can be identified in rectal mucosa at diagnosis whose expression will accurately predict week 52 SFR.

4.3. Exploratory Hypothesis

Hypothesis 11. The prediction model of hypothesis 5 for week 52 SFR can be further improved by incorporating additional genetic, genomic and immune factors.

5. STUDY ENDPOINTS

5.1. Overview of Primary and Secondary Clinical Endpoints

The primary clinical endpoint is being in corticosteroid free remission (SFR) at 52 weeks on mesalamine only without prior recourse to medical or surgical rescue therapy. The proportion of patients in SFR at 52 weeks will serve as the primary analysis variable. Secondary endpoints include SFR at other time-points, endoscopic outcomes at week 52, quality of life measures at 52 weeks, and colectomy during follow-up. For endpoints ascertained at a particular time-point, the proportion of patients having the endpoint will serve as the principal analysis variable. For colectomy, time to colectomy will serve as the principal analysis variable.

5.2. Clinical Endpoints

5.2.1. Primary Endpoint: SFR at 52 Weeks on Mesalamine Only

The primary efficacy endpoint is being in SFR (PUCAI<10) at 52 weeks from start of therapy while receiving mesalamine only as maintenance therapy without the need for rescue therapy with IM, CI, anti-TNFα, or surgery.

5.2.2. Secondary Endpoints

The secondary endpoints are defined relative to start of therapy:

- PUCAI <10 at 4 weeks
- SFR at 12 weeks without the need for rescue therapy
- SFR at 26 weeks without the need for rescue therapy
- SFR at 104 weeks without the need for rescue therapy
- Endoscopic response (Mayo score reduced by ≥1) and being 0,1 at week 52
- Endoscopic remission (Mayo score 0) at week 52
- IMPACT – III at 52 and 104 weeks
- Colectomy free status during the follow-up period

5.2.3. Definitions of Corticosteroid (CS) Status

CS-free: Subjects will be considered to be CS-free if they are taking no CS at the assessment and have been CS-free for a minimum of 2 weeks at that point. Alternate day CS will not be considered CS-free.

CS responsive: Clinical improvement and termination of CS within 12 weeks of initiating therapy, and remaining off CS for the remainder of the first year for a minimum of six months following initial tapering before requiring re-treatment with CS.

CS dependent: Required CS >12 weeks to control symptoms, or at least one additional course of CS starting <6 months after initial successful CS weaning

CS refractory: Unresponsive to oral CS within four weeks of starting therapy and required rescue therapy with CI or anti-TNFα therapy; or required rescue therapy with CI or anti-TNFa

therapy because of failure to respond to intravenous CS, or required colectomy because of failure to respond to CS.

6. EXPERIMENTAL DESIGN AND METHODS

6.1. Study Subjects and Eligibility

A total of up to 475 children and adolescents will be recruited after being newly diagnosed with ulcerative colitis. We anticipate that patient recruitment will take place over the initial 4 years of the study. Patient follow-up will be for a minimum of one year, and will continue until the termination of the study allowing for up to 1 to 5 years of observation.

6.1.1. Eligibility

The eligibility criteria are defined to reflect the typical patient population with ulcerative colitis for whom initial therapy with either mesalamine or corticosteroids followed by mesalamine is considered standard of care. Patients with proctitis, who only represent approximately 5-7% of pediatric patients with ulcerative colitis are not eligible for study, as they are generally managed with topical and not oral therapy.

6.1.1.1. Inclusion Criteria

- Age ≥ 4years and ≤17 years at initiation of therapy (achieved 4th birthday, not yet 18th)
- Weight ≥15 kg
- New diagnosis of ulcerative colitis established by standard clinical, endoscopic, and histologic features at the PROTECT study site (see Case Ascertainment below)
- Colitis extending beyond the rectosigmoid (Paris classification E2, E3, or E4)[144]. If a patient is seriously ill and the clinician does not advance the colonoscope beyond the sigmoid colon but the clinical condition of the patient highly suggests more extensive disease then that patient is eligible for study.
- Disease activity by PUCAI of ≥10 at diagnosis
- No therapy previously initiated to treat the newly diagnosed ulcerative colitis
- Stool culture negative for routine enteric pathogens (Salmonella, Shigella, Campylobacter, *E. coli 0157:H7*) and *Clostridium difficile* toxin. Recent successful treatment for *Clostridium difficile* does not exclude a patient if toxin now absent. However, the patient must be a minimum of 5 weeks from the time treatment was started at the time toxin is absent.
- Stool study negative for enteric parasites (ova and parasites)
- Parent/guardian consent and patient assent
- Ability to remain in follow-up for a minimum of one year from diagnosis
- Female patients of child bearing age must have a negative urine pregnancy test and practice acceptable contraception (e.g., abstinence, intramuscular or hormonal contraception, two barrier methods (e.g., condom, diaphragm, or spermicide), intrauterine device, verbal report of the partner with history of vasectomy, or be surgically sterile). All female patients of childbearing potential (post-menarche) will undergo urine pregnancy testing at screening and must not be lactating.

6.1.1.2. Exclusion Criteria

- Clinical, endoscopic, radiologic, or histologic evidence suggesting CD consistent with Paris and NASPGHAN criteria[144, 145], (see Case identification below)
- A previous diagnosis of inflammatory bowel disease for which treatment was given
- Evidence of any active enteric infection at the time of study entryUse of any oral CS for non-gastrointestinal indication within the past 4 weeks (e.g., asthma). Use of inhaled CS does not exclude a patient.
- History of use of IM or anti-TNFα agent for other medical conditions (e.g., juvenile rheumatoid arthritis) within the past 6 months
- Use of Accutane within the past 4 weeks
- Use of any investigational drug within the past four weeks
- Use of any 5-aminosalicylate within the past 4 weeks
- Pregnancy
- Subjects with poorly controlled medical conditions (e.g. diabetes, congestive heart failure)
- Proctitis or proctosigmoiditis only (Paris classification E1) on colonoscopic evaluation
- Chronic renal disease (BUN and serum creatinine >1.5 times the upper normal limit)
- Hepatic disease (AST or ALP greater than 3 times the upper normal limit in the absence of concomitant liver disease associated with IBD following full evaluation)
- History of allergy or hypersensitivity to salicylates, aminosalicylates, or any component of the Pentasa capsule.
- History of coexisting chronic illness or evidence of significant organic or psychiatric disease on medical history or physical examination, which, in the Investigator's opinion, would prevent participation in the study
- History or presence of any condition causing malabsorption or an effect on gastrointestinal (GI) motility, or history of extensive small bowel resection (greater than half the length of the small intestine).
- The finding of Helicobacter pylori at the time of evaluation does not exclude the patient from the study. Whether to treat this patient for Helicobacter pylori and when will be left to the discretion of the site.

6.2. Case Identification and Consent

Subjects will be recruited from the clinical population served by up to 30 high volume pediatric IBD centers in the United States and Canada.

6.2.1. Case Identification

Patients with a confirmed or suspected diagnosis of UC are eligible for enrollment. All patients must be newly diagnosed at a PROTECT study site. Investigators will have a checklist form for each potential subject to confirm eligibility. A diagnosis of UC for this study will require [145]:

1) Clinical history consistent with colonic inflammation (e.g. any combination of diarrhea, bleeding, abdominal pain)
2) Negative culture for enteric pathogens as noted in Inclusion/Exclusion criteria
3) Endoscopic findings of diffuse continuous mucosal inflammation involving the rectum and extending to a variable degree proximally. Features may include granularity, loss of vascular pattern, friability, small superficial ulcers, mucopurulent exudate, and a line of demarcation between abnormal and normal colon in a patient whose colitis does not extend to the cecum. Histologic features of chronicity must be present. There will be cryptitis and/or crypt abscesses to demonstrate activity. Chronicity will be demonstrated by mucin depletion, crypt distortion, crypt branching, crypt atrophy, basal lymphocytosis (not all features need be present).
4) Assessment of the small bowel is not required to establish an initial diagnosis of UC. However imaging of the small bowel via barium contrast, magnetic resonance enterography, CT enterography, or capsule endoscopy is generally part of standard evaluation of all newly diagnosed children with inflammatory bowel disease and will be performed during the initial 3 months after diagnosis. Visualization of the terminal ileum during colonoscopy may substitute for small bowel imaging at the discretion of the investigative site but the site will be encouraged to do additional imaging as noted above.

Non classic finding of UC that **do not** exclude a diagnosis of UC include[145]:

- Gastritis without distinct aphthous lesions (non specific gastritis)
- Backwash ileitis in the presence of pancolitis (ileal erythema without ulceration)
- Periappendiceal inflammation in a patient without pancolitis (cecal patch)
- Rectal inflammation less severe than more proximal colon (relative rectal sparing)
- Microscopic ileitis without granuloma
- Microscopic gastritis without granuloma
- Macroscopic patchiness (macroscopically normal colonic mucosa between two areas of colonic inflammation)
- Serological reactivity to anti-microbial antigens (ASCA, CBir, OmpC, I2).

5) A diagnosis of UC will be EXCLUDED if any of the following are present:

- Absolute histologic rectal sparing
- Perianal fistula, atypically located fissures, or significant skin tags
- Stenosis, cobble stoning, or significant linear ulceration in the terminal ileum
- Segmental colitis i.e. discontinuous disease: macroscopic and microscopic skip lesions
- Radiologic evidence of stricture or fistula
- Histopathology showing transmural inflammatory cell infiltrate (noted at colectomy and in the absence of fulminant disease) or epithelial granulomas not related to crypt rupture (at any time) and absence of identifiable infectious agents. Transmural inflammation might be established at a time after diagnosis and would subsequently establish a diagnosis of CD

Capsule endoscopic lesions that are considered indicative of CD by the treating physician or MRI/CT enterography demonstrating disease in the small bowel consistent with CD or barium contrast series indicating CD.

6.2.2. Consent

The parents or legal guardians of children suspected of having ulcerative colitis will be approached for consent for enrollment into the study. Assent will also be obtained from the patient when appropriate based on age and institutional IRB requirements.

6.2.3. Ethical Considerations

Prior to initiating enrollment at each clinical site, the informed consent document will be reviewed by the Steering Committee, NIDDK Project Office, Data and Safety Monitoring Board (DSMB), and the Institutional Review Board (IRB) at each clinical site to ensure that the document is consistent with study protocol, federal regulations, and individual institutions' policies. Enrollment will not commence in the study as a whole until this document has been approved by the Steering Committee, DSMB, and the NIDDK Project Office. In addition, enrollment will not commence at a site until approval is granted by that site's IRB.

6.2.3.1. Special Ethical Issues

Genetics

PROTECT will be collecting specimens for and conducting analyses of genetic information on all participants. Strict confidentially standards are in place and will be maintained to protect the privacy of PROTECT participants. Biospecimen samples will be labeled with a unique identifier that does not contain any protected health information or otherwise identifies a participant.

In the event a new genetic marker is found during the course of PROTECT, participants with this hypothetical marker will not be notified. The analyses conducted as part of PROTECT are not intended to be diagnostic, and may not take place in a Clinical Laboratory Improvement Amendments (CLIA) certified lab.

Consenting for use of genetic material will be over and above consenting to participate in the study. A patient may participate in the study without consent being given to analyze genetic information.

Specimen Storage for Future Studies

Some of the biospecimens collected as part of this study will be stored for future analyses. These specimens will not be individually identifiable by the specimen repository, laboratory or Data Coordinating Center (DCC) personnel. The DCC will develop and maintain a tracking system whereby study participants can modify their level of consent. Participants can ask that any specimens still in storage be destroyed and not included in future analyses. The request to remove stored specimens will be made at the clinical sites to preserve participant confidentiality.

Week 52 Flexible Sigmoidoscopy

All patients will undergo a diagnostic colonoscopy prior to study entry. This is part of the standard work-up for diagnosing ulcerative colitis and is not considered to be a study procedure. An optional repeat flexible sigmoidoscopy will be offered to study subjects at week 52 who are in clinical remission and receiving mesalamine only to assess for mucosal healing. Consent for the week 52 sigmoidoscopy will be separate from consent for the study and patients may refuse the week 52 sigmoidoscopy without jeopardizing either their continued participation in the study or the medical care they receive.

Additional clinically indicated colonoscopy
If a patient undergoes a colonoscopy during the study course that the attending clinician deems needed for clinical care decisions then consent will be sought to obtain 4 additional rectal mucosal biopsies for study purposes. Consent for this additional biopsy material will be separate from consent for the study.

6.2.4. Risks and Benefits to Participants

6.2.4.1. Potential Risks at Clinical Sites

Blood Draws: Phlebotomy can cause discomfort, bruising, a small risk of infection, or thrombosis. Standard aseptic technique will be employed to prevent infection. All blood draws will take place at the time of routine blood test monitoring.

Stool Sample: There is no specific risk to the subject in obtaining a stool sample though bringing a stool sample from home may be inconvenient.

Rectal biopsy: The procurement of four additional rectal biopsies from the proximal to mid rectum by grasp forceps at the time of diagnostic colonoscopy or during a follow-up clinically indicated colonoscopy engenders minimal additional risk to the patient. The risk of perforation is largely related to the colonoscopy itself, not mucosal biopsies [146].

Flexible sigmoidoscopy at 52 weeks: Risks of flexible sigmoidoscopy include making a hole in the lining of the colon which would need to be surgically repaired (perforation); bleeding; and infection. Each of these risks is quite low. The risk of colon perforation is approximately 1 out of every 1000 patients and may not be related to the biopsies. If subjects agree to additional biopsy samples for research, obtaining the additional colon biopsy samples may not significantly increase the risk of perforation, bleeding, or infection associated with the colonoscopy. The subject would not ordinarily have the week 52 sigmoidoscopy as part of regular medical care unless there were a medical indication or in the view of the attending physician it was needed.

Questionnaire data collection: All measures have been used in previous research, without any apparent significant risk. It is feasible that subjects may experience some anxiety or distress associated with completion of the questionnaires; however this is unlikely to result in severe or ongoing effects. All project staff will be extensively trained on administration of instruments, with emphasis on preventing and dealing with anxiety or discomfort. If, as a function of the assessment, a significant mental health risk is identified, parents will be informed and an appropriate referral for treatment will be made with parental consent. If imminent danger is noted the patient will be escorted to a local emergency room or mental health facility for additional evaluation. More information about the questionnaires is provided later in sections 6.5-6.7.

Genetic Testing: A breech in confidentiality could have potential unforeseen effects, and potentially could affect insurability. Every effort will be made to maintain confidentiality by identifying samples by a laboratory sample number rather than by personal identifier, and keeping all study data in locked files as noted above.

Treatment protocols: The treatment protocols have been determined by consensus of pediatric inflammatory bowel disease experts and are considered to represent the standard of care for children and adolescents with ulcerative colitis. This standard of care therapy would therefore not be expected to engender any risks of therapy that would exceed normal clinical practice at any of the investigative sites. The potential risks and benefits of mesalamine and prednisone or liquid equivalent prednisolone will be discussed with each patient and family in the customary fashion. For patients requiring escalation of therapy including a rescue medication or surgery, each investigative site will use its customary explanation of risks and benefits of each therapy, and the determination of which rescue therapy to be used will be at the discretion of the attending physician per their normal standard of care.

Breach of confidentiality: There is a risk that someone not authorized to view participant information, including identifiable information, will gain access to this information. Clinical sites will take measures to prevent this from happening and will comply with local regulations.

6.2.4.2. Adequacy of Protection Against Risks at Clinical Sites

Recruitment and Informed Consent: Study participants will be identified based on a suspected new diagnosis of ulcerative colitis, and will be approached by research staff. Research staff will explain the study, answer questions, and obtain informed consent and assent as indicated. Informed consent/assent will be documented by the parents' and child's signature on the appropriate form and a signature of the person obtaining the consent. A copy of the signed form will be returned to the family.

Protection Against Risk: Every effort will be made to maintain confidentiality. Study records and samples will be recorded by a numerical identifier. The list of names associated with the identifying numbers will be kept separate from the questionnaires in a folder labeled in such a way that its meaning or significance will not be evident to anyone not involved in the study. Subjects will be informed in advance of the voluntary nature of the study. Previously consented individuals have the option to opt out of this study at any time.

We have devised several layers of protection for Protected Health Information (PHI). All information entered into the PROTECT study database is stored on a secure server and the database is password protected. Access to the database is restricted to those directly involved in data collection or analysis. Each individual is given a unique identifier in the database. The PHI is stored separately from the clinical information, laboratory values, and genotype data, and is not available to laboratory personnel.

6.2.4.3. Potential Risks at the Data Coordinating Center

The only direct risk to participants originating from the DCC is the risk of breach of confidentiality. The DCC has many safeguards against this, including staff training, controlled access to computer and paper files, back-up systems for electronic data, and protection against malicious code. More detail regarding the DCC's security plans and measures is available in Section 10 of this protocol: Data Management.

6.2.4.4. Change in or Loss of Insurance Coverage

There is a risk that a participant may experience change in coverage, change in cost, or loss of insurance as a result of incidental findings during the course of the study. Treatments provided and some of the tests conducted are part of routine clinical care and will be billed as such. Other tests that are performed specifically as part of the PROTECT study are for research purposes only, and are not for clinical or diagnostic use. Therefore, the results of these tests will not be reported to insurance companies.

During the course of a research-related procedure, it is possible that a previously unknown disease or illness will be discovered or that a change in disease severity will be found. If one of these two events occurs, the participant may be referred to care outside of the PROTECT study. The resultant diagnosis and care may result in changes to a participant's insurance.

6.2.4.5. Potential Benefits

This study may not result in any direct benefit to participants. However, results of this study will help determine response and remission rates to standardized treatment protocols, and potentially identify clinical, immunologic, or genetic factors that are associated with disease that requires escalation of therapy to include rescue medications or surgery facilitating future clinical trials in ulcerative colitis. Subjects will be paid a small stipend for biospecimens and Pentasa (mesalamine) will be provided free of charge for up to one year from initiation of Pentasa therapy.

Importance of the Knowledge to be Gained: This study is the first step in establishing baseline knowledge of response and remission to standardized care in the treatment of UC in children and potentially help build predictive models of response. This information will be invaluable in designing future clinical trials, particularly in children who are deemed to be at high risk for requiring escalation of therapy and use of rescue medications or possible colectomy. The additional risk to participants is minimal compared to usual clinical practice as they will receive standardized care designed by experts in pediatric IBD and then will utilize all additional available therapies as their disease course warrants. Given the minimal risk to participants, as well as the potential benefit to future children with UC, we believe the study is justified.

6.2.5. When and How to Withdraw Participants from Therapy or Assessment

6.2.5.1. Change in Diagnosis

A participant will be withdrawn from the study if there is a change in his/her diagnosis to CD..

6.2.5.2. Principal Investigator (PI) Discretion

A participant can be withdrawn from the study if the clinical site PI determines that it is the best interest for the subject to stop participation in the study or that it is unsafe or unethical to continue in the study.

6.2.5.3. Participant Request

Participants may withdraw at any time for any reason. At the time of withdrawal participants can either 1) decline to provide any more data or specimens to the study but allow use of previously collected data and/or specimens, 2) withdraw all their data from study databases and request that any stored samples be destroyed, or 3) withdraw some portion of the data collected (i.e., participants may withdraw specimens but not examination data or vice versa).

6.2.5.4. Participant Exit Interview

If a participant chooses to withdraw from the PROTECT study, the study coordinator will endeavor to conduct an exit interview to determine the disposition of the participant's study data. The participant may decline to participate in the exit interview, in which case the consent/assent in place at the time of study withdrawal will be used to determine the status of the participant's data.

6.3. Baseline and Follow-up Evaluation

The schedule of study visits and assessments is presented in Table 6.1. "Baseline" may consist of two separate visits, one for diagnosis and another to begin treatment. A maximum of 14 days will be allowed between diagnosis and start of treatment. **The beginning of treatment will be regarded as day 0 for all subsequent milestones.** Follow-up clinic visits will be conducted for a minimum of 1 year to a maximum of 5 years following study enrollment depending upon the actual time of enrollment. Demographic and contact information will be collected at baseline. Several assessments will be made at baseline and at each study visit. These include PUCAI scoring, and completion of behavioral and quality of life instruments. Adherence to mesalamine therapy will be assessed at post-baseline visits. Biospecimens will be collected according to the schedule in the table. The Mayo endoscopy sub-score will be determined from the diagnostic colonoscopy and in those patients qualifying for and agreeing to the week 52 sigmoidoscopy.

Various assessments will also be made through telephone contacts between study visits, including response to treatment, and adherence to medication.

Table 6.1. Schedule of visits and assessments#

Activity	Baseline	2, 6, 8, 10 weeks – telephone assessment #	4 ¤ weeks	12α weeks	26α weeks	39α weeks	52α weeks	78α weeks	104α weeks	**Annual visits: 1-5 years
Routine Blood Monitoring*	X§		X	X	X	X	X		X	X
Study Specific Blood	X		X	X			X			
Urinalysis#	X				X		X		X	X
Rectal biopsies	X						X			
Stool sample	X		X	X			X			
Questionnaire$	X	Up to	X				X		X	
PUCAI	X	X	X	X	X	X	X	X	X	
Adherence monitoring	X	X	X	X	X	X	X			
Colectomy free status		X	X	X	X	X	X	X	X	X
Height/Weight	X		X	X	X	X	X	X	X	X

Visit timing is scheduled relative to start of therapy. Additional phone calls (2,6,8,10 weeks) may occur during the first 12 weeks of therapy for routine patient contact and oral corticosteroid adjustment as necessary. It is expected that all patients will receive a week 2 phone call.

* Routine blood monitoring for standard of care for children with ulcerative colitis includes: complete blood count with differential, erythrocyte sedimentation rate, serum albumin, C-reactive protein, serum aminotransferases, serum gammaglutamyl transferase (GGT) and renal function studies (BUN, creatinine). Serum amylase and lipase are also monitored in patients on mesalamine therapy over the first 12 weeks of therapy. Patients on mesalamine will have yearly urinalysis.

§ Baseline routine blood monitoring should be done as close to Day 0 as possible, and no longer than 14 days previously in a clinically stable patient

α Follow-up routine blood monitoring should be done as close as possible to the designated time point when deemed necessary for clinical care by the attending physician

** Follow-up visits beyond 1 year will depend upon time of enrollment in the study.

$ Questionnaire will be completed between baseline and 4 weeks, and then again at 52, 104 weeks.

6.4. Treatment

Details of the standardized PROTECT treatment regimen are provided in section 7 of this protocol.

6.5. Pediatric Ulcerative Colitis Activity Index (PUCAI) [2]

The Pediatric Ulcerative Colitis Activity Index (PUCAI) is a key component of primary and secondary outcomes of PROTECT as well as guiding treatment. PUCAI scoring for study outcome data will be done by a PROTECT Study physician and will be based on information

they have directly obtained during clinical evaluation of the patient that visit. Study nurses or coordinators may obtain a PUCAI score by patient report in a bi-weekly telephone follow-up as part of steroid tapering. The scores obtained by the Study Nurse/Coordinator will be based on patient self-report and may be used to adjust treatment but will not be utilized for determination of study outcomes.

6.5.1. PUCAI Determination

The PUCAI is scored using Table 6.2. The range of possible scores is 0-85 and the classification within this range is:

PUCAI<10	Inactive disease
PUCAI 10-34	Mild activity
PUCAI 35-64	Moderate activity
PUCAI ≥65	Severe activity

6.5.2. Justification for the Use of PUCAI

The PUCAI is now considered the standard assessment tool for UC in children [2, 147, 148]. It has been validated in large numbers of patients both during its initial development and subsequently in real world experience in the Pediatric IBD Collaborative Research Group Registry. A recent sub-analysis of the data originally published previously [2] demonstrated no difference in validity between groups ≤12 years and >12 years as seen in Table 6.3. Moreover, age (entered as a continuous variable) was not associated with the PUCAI score in a multivariate analysis in which colonoscopic score was the dependent variable and age and the PUCAI score as the explanatory variables (beta=1.17, $p<0.001$ for the PUCAI and beta=–0.146, $p=0.26$ for age).

Data to support cut scores for defining various levels of disease activity and changes in level of disease activity have been validated previously [2]. The sub-domains of the PUCAI have been validated as appropriate, comprehensive, and able to detect change in patient condition. The major strength of the PUCAI lies in its high psychometric properties (high reliability, validity, and responsiveness). A recently published study demonstrated that a physician based index is required to adequately assess disease activity in children with UC [149]. The study data demonstrated that disease rating by physicians (using the PUCAI) more closely relates to measures of disease activity than did patients' rating of symptoms, regardless of the patient's age.

Table 6.2. PUCAI Scoring [2]

ITEM	POINTS
1. Abdominal pain:	
No pain Pain can be ignored Pain cannot be ignored	0 5 10
2. Rectal Bleeding	
None Small amount only in less than 50% of stools Small amount with most stools Large amount (>50% of the stool content)	0 10 20 30
3. Stool consistency of most stools	
Formed Partially formed Completely unformed	0 5 10
4. Number of stools per 24 hours	
0-2 3-5 6-8 >8	0 5 10 15
5. Nocturnal stools (any episode causing awakening)	
No Yes	0 10
6. Activity level	
No limitation of activity Occasional limitation of activity Severe restricted activity	0 5 10
SUM OF PUCAI (0-85)	**0-85**

Table 6.3. Correlations between constructs of disease activity and the PUCAI stratified by age groups (numbers represent rho coefficient)

	≤12 years	>12 years old
Colonoscopic score #	0.83**	0.72**
Mayo score	0.93**	0.95**
CRP	0.45*	0.54*
Physicians' global assessment of disease activity	0.92**	0.91**

#Scored according to Beattie et al [150] in each part of the colonic segment, as previously described [151].

*P<0.01 **P<0.001

6.6. Mayo Sigmoidoscopy Score

Endoscopic severity will be assessed at study entry in all study subjects and in the subset of patients who qualify for and agree to a repeat week 52 sigmoidoscopy using the Mayo endoscopy score [1]. Photographs corresponding to each score will be provided to investigative sites to serve as a guide. This scoring system is summarized in Table 6.4. A de-identified photo of the most inflamed segment of the rectosigmoid will be electronically recorded and labeled with the patient's unique study identification number and transmitted to the DCC where it will also be read by central readers. A centralized histologic evaluation of a rectal biopsy that assesses inflammation (acute, chronic, severity) as well as architectural changes (crypt distortion/atrophy, surface villiform changes, basal plasmacytosis, and Paneth cell metaplasia) will also be performed from each endoscopic evaluation and recorded on a case report form.

A recent study in which four expert endoscopists determined the Mayo score from images obtained from 93 UC patients demonstrated very good intra- and inter-observer agreement for the Mayo score (kappa value of 0.75 and 0.74, respectively) [152].

Table 6.4. Schroeder endoscopic scoring system (Mayo score)

0 = Normal or inactive disease
1 = Mild disease (erythema, decreased vascular pattern, mild friability)
2 = Moderate disease (marked erythema, absent vascular pattern, friability, erosions)
3 = Severe disease (spontaneous bleeding, ulceration)

6.7. Other Assessments

6.7.1. Quality of Life Assessment

Study subjects (ages 9-17 years) will complete a standardized quality of life instrument (IMPACT-III) [153] between initiation of standardized therapy and their 4 week visit, at 52 weeks, and at 104 weeks. A score of 144 or greater will be used as indicative of a good quality of life. The instrument has been validated in pediatric patients from age 9 to 17 with inflammatory bowel disease, and the results will serve as a secondary endpoint [153]. These

data will be also used in an ancillary analysis of the effects of behavioral factors, adherence, and clinical disease severity upon quality of life.

6.7.2. Behavioral Assessment

In order to account for behavioral variables that may affect medication adherence and thereby treatment outcome, the Behavior Assessment System for Children – Second Edition (BASC-2) will be used in this trial [154]. The age appropriate self- or parent-report version of these measures will be completed by patients and parents between initiation of therapy and the 4 week visit and at 52 weeks, and at 104 weeks. The BASC-2 is a widely used, reliable (α = 0.90 – 0.94, test-retest r = 0.84 – 0.90 for parent report; α= 0.94 – 0.96 , test-retest r= 0.81 – 0.82 for child self-report), and valid inventory to assess and identify children and adolescents (ages 2-18; different forms for developmental levels) with emotional disturbances and behavioral disorders. The BASC-2 measures externalizing, internalizing, and school problems, adaptive skills, and other problems. Both the Parent Rating Scale and Child Self-report forms will be used. Respondents rate the presence and frequency of behavioral symptoms via a four-point Likert scale and by answering several true/false questions. Computerized scoring provides subscale T-scores. Elevated T-scores indicate clinically significant pathology in the particular domain (e.g., internalizing problems) compared to norms. These data will be used in an ancillary analysis of the effects of emotional disturbances and behavioral disorders on adherence.

6.7.3. Adherence

For this trial, the Medication Event Monitoring System (MEMS®) will be used for adherence measurement. The MEMS® 6 Trackcap monitoring system includes a standard plastic vial and cap containing a microelectronic circuit that records each time the cap is removed. Adherence data parameters include number and percentage of doses removed, optimal daily dosing, estimation of therapeutic coverage, and mean and range of interdose intervals. Research using these electronic monitors has demonstrated excellent validity, with 93% predictive value, 82% sensitivity, and 89% specificity. Data from these devices are transferred to computer software that generates graphic feedback for patients to view. MEMS monitoring can start at any time up to and including the Week 4 visit. At study visits at weeks 4, 12, 26, 39, and 52 data will be downloaded from the cap. Pill counts and self-report adherence data will also be obtained in conjunction with study visits and electronic adherence data collection. These additional adherence assessments will be used for supplementary data (e.g., mechanical failure of MEMS devices, family forgets to bring MEMS device to study visit for data download). In the (rare) event of missing electronic monitor data, we will use pill count data to impute an adherence percentage during the missing time point. We will use www.mymedschedule.com to utilize either text messaging or email reminders when possible

6.8. Collection of Laboratory Samples

6.8.1. Blood and Stool

Blood and stool will be collected at clinically indicated specified time points during a patient's disease course. "Standard of Care" laboratory testing including complete blood count, erythrocyte sedimentation rate, C-reactive protein, serum albumin, blood urea nitrogen, creatinine, serum aminotransferases, and gammaglutamyl transferase (GGT) will be measured

at diagnosis, 4 weeks, 12 weeks, 26 weeks, 39 weeks, 52 weeks, 104 weeks and will be performed at the Clinical Core Laboratory facility of the enrolling institution at diagnosis. Any significant laboratory abnormality detected will be followed up with subsequent testing as clinically indicated until resolution. "Non-Standard" testing will be shipped to a central facility at Emory University School of Medicine, the repository for biospecimens of the PRO-KIIDS research group. A summary of the biospecimens which will be collected, their specific use for the PROTECT study, and their potential future use for ancillary studies, is provided in Table 6.5.

Blood draws will be performed by qualified nurses, physicians and phlebotomists according to standard phlebotomy techniques. Participants will be asked to collect a stool sample at baseline (i.e., prior to the commencement of induction therapy) and at week 4, week 12, and week 52.

Table 6.5. Blood and Stool Sample Collection and Usage

Biospecimen	Entry	Week 4 (Day 30)	Week 12	Week 26	Week 39	Week 52	PROTECT Data	Ancillary Studies
Blood								
Standard of Care	X	X	X	X	X	X		
Study Specific	X	X	X			X	Vitamin. D Serology	Multiplex Cytokines Proteomics Metabolomics
Genomic DNA	X					X	SNP Chip	Fine mapping of genes associated with SFR Patient epigenetics
Mucosal Biopsy								
Colon DNA	X					X	None	Microbiome Patient epigenetics
Colon RNA	X					X	RNA-seq by week 52 SFR	Multiplex PCR
Stool								
Study Specific	X	X	X			X	Calprotectin OPG	Microbiome Metabolomics

Timing of biospecimen collection is relative to start of initial therapy

6.8.2. Mucosal Biopsies

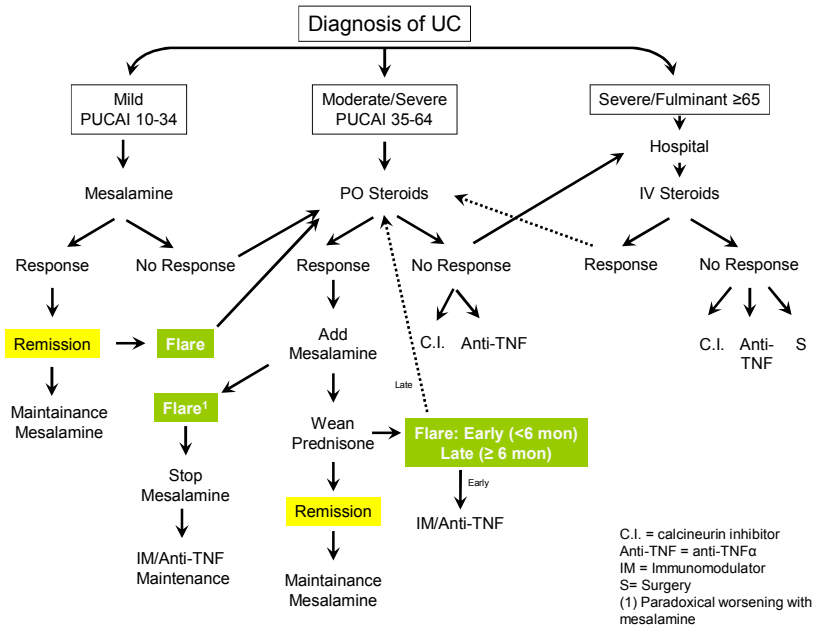
Four (4) rectal biopsies will be obtained at the time of diagnostic colonoscopy from the proximal to mid rectum for research purposes along with routine biopsies used to establish a diagnosis. This will include all subjects at entry, and the subset of subjects who qualify for and agree to a week 52 sigmoidoscopy, or those who consent for study biopsy during additional routine colonoscopy..

7. TREATMENT REGIMEN

7.1. Overview

An overview of the treatment regimen is presented graphically in Figure 7.1.

Figure 7.1. Graphical Representation of Treatment Regimen



7.2Mesalamine

Mesalamine (Pentasa®) has been chosen as the aminosalicylate for this study because of ease of administration to children of all ages in clinical practice over the past 10-15 years, ease of standardization of dosage, previous adult studies showing safety and efficacy [15, 17] and lack of oral staining that is associated with balsalazide. Additionally, it can be administered to children unable to swallow the capsule by opening up and mixing with apple sauce or yogurt-like foods. The investigators have obtained an IND (111863) for this study. Mesalamine will be provided by Shire Pharmaceuticals in 500 mg capsules that will be distributed to study sites. The package insert describing properties of the drug as well as potential toxicity is in the Appendix attached to this protocol. This medication will be provided free of charge to each patient participating in this clinical protocol until the 52 week outcome assessment or until it is discontinued prior to 52 weeks.

7.3. Initiation of Medical Therapy

The dosing schedule of mesalamine is shown in Table 7.1. Pentasa®) comes in 500mg capsules, and doses will need to be rounded to the nearest 500mg increment, with a maximum dose of 75 mg/kg/day. The average dose for the pediatric population will be approximately 70 mg/kg/day. Patients will be allowed to escalate to the final dose over 4 days to minimize side-effects such as headache.

Table 7.1. Reference 5-ASA Total Daily Dosing

Weight Range	5-ASA Daily Dose Rounded to the nearest 500 mg, max. 4 g	5-ASA Daily Dose Range (mg/kg) Up to 75 mg/kg/day	
		Min	Max
15 – 19.9 kg	1000 mg	50 mg/kg	66 mg/kg
20 – 26.4 kg	1500 mg	57 mg/kg	75 mg/kg
26.5 – 32.9 kg	2000 mg	61 mg/kg	75 mg/kg
33 – 39.9 kg	2500 mg	63 mg/kg	75 mg/kg
40 – 46.4 kg	3000 mg	65 mg/kg	75 mg/kg
46.5 – 52.9 kg	3500 mg	66 mg/kg	75 mg/kg
53+ kg	4000 mg		75 mg/kg

7.3.1. Ambulatory patients with mild disease at diagnosis (PUCAI 10-34)

Initiate treatment with mesalamine (Pentasa®) in two divided doses. If PUCAI is not <10 within 4 weeks, or if patient worsens (PUCAI ≥ 35) on mesalamine then prednisone or liquid equivalent prednisolone is instituted and mesalamine is stopped. Prednisone dosing schedule will be as noted below. Mesalamine will be added back to regimen after 2 weeks on prednisone if patient has responded (20 point decrease in PUCAI and PUCAI<34). The tapering schedule of prednisone will then be conducted per protocol to allow possible discontinuation of prednisone by week 10. If PUCAI worsens (increases by 20 or more points) upon re-introduction of Pentasa then consider paradoxical response to Pentasa and need for rescue therapy.

7.3.2. Ambulatory patients with moderate to severe disease (PUCAI≥35)

Initiate treatment with prednisone or prednisolone, 1-1.5 mg/kg/day, (maximum 40-60 mg in a single morning dose, either as tablet or liquid equivalent), rounded up to the nearest 5 mg value. This dose will be continued for 2 weeks to assess response. If the patient responds (PUCAI decrease of at least 20 points with resulting PUCAI ≤ 34, and steroids administered for at least 2 weeks), mesalamine will be added and the initial prednisone or liquid prednisolone equivalent dose continued for one more week to allow for observation for paradoxical worsening with the mesalamine. The mesalamine (Pentasa®) will be given as per Table 7.1. There are some patients in whom the PUCAI is in the 35-50 range in whom the clinician feels that initial CS therapy is not warranted. For those patients an initial trial of Pentasa is allowable. However, if a patient does not respond and then requires prednisone or liquid prednisolone equivalent they should enter the standardized CS pathway.

7.3.3. Hospitalized patients with severe UC (PUCAI≥65)

Treatment with intravenous CS is instituted after stool cultures/toxin assays reveal no enteric pathogen. If intravenous CS therapy is used for ≤14 days and the patient is discharged at that point, then discharge CS will be given in the same manner as for ambulatory patients with moderate to severe disease. Addition of mesalamine would then take place as noted above.

Patients with fulminant disease at diagnosis who receive intravenous CS for more than 14 days, or those who require rescue with infliximab, CI, or surgery while hospitalized, will be eligible for recording of clinical outcomes and the biomarkers sub-study but will not enter the oral standardized treatment protocol. They will be considered treatment failures with respect to the study primary outcome. These patients will be considered part of the up to 475 patients enrolled in this study.

7.3.4. CS Tapering Schedule

The initial oral prednisone or liquid equivalent prednisolone dose will be maintained for at least three weeks (including the intravenous CS phase if patient initially hospitalized for ≤7 days) and for at least 7 days after the introduction of mesalamine. Mesalamine will be added after a minimum of 2 weeks of steroid therapy and once the PUCAI is ≤34. This will allow for a period of observation to ensure no paradoxical worsening by the mesalamine occurs prior to tapering the prednisone or liquid equivalent prednisolone . Prednisone or liquid equivalent prednisolone dose decreases will then be contingent upon adequate clinical improvement, defined by a decrease in PUCAI of ≥20 points from initial PUCAI with the total score being ≤ 34 points and the clinician deeming tapering appropriate. The goal will be to taper so that prednisone or liquid equivalent prednisolone is stopped by week 10 to allow evaluation of corticosteroid-free status at the 12 week endpoint (that is, off prednisone for 2 weeks). Prednisone can be weaned according to the schedule in Table 7.2.

Table 7.2. CS tapering schedule. All doses are in mg/day prednisone equivalent

Week 0-1	Week 1-2	Week 2-3	Week 3-4	Week 4-5	Week 5-6	Week 6-7	Week 7-8	Week 8-9	Week 9-10	Week 10-11	Week 11-12
60	60	60	50	40	30	20	15	10	5	0	0
50	50	50	40	30	25	20	15	10	5	0	0
45	45	45	40	30	25	20	15	10	5	0	0
40	40	40	35	30	25	20	15	10	5	0	0
35	35	35	30	30	25	20	15	10	5	0	0
30	30	30	25	20	20	15	15	10	5	0	0
25	25	25	20	20	15	15	10	5	5	0	0
20	20	20	15	15	12.5	10	7.5	5	2.5	0	0
15	15	15	12.5	12.5	10	7.5	7.5	5	2.5	0	0

Criteria for not weaning prednisone or liquid equivalent prednisolone at weekly milestone: If the PUCAI increases from the previous week (but <20 points) the prednisone or liquid equivalent prednisolone dose will not be weaned. Alternative criteria for not weaning based on text above would be: PUCAI score has increased and is no longer ≥20 points lower than baseline PUCAI or is no longer <34. The attending physician will have the discretion to attempt to bring a patient back into the above weaning schedule if there was a temporary increase in PUCAI. The final decision whether to wean the prednisone or liquid equivalent prednisolone dose will be contingent upon the physician's discretion that weaning is appropriate.

Criteria for increasing prednisone or liquid equivalent prednisolone therapy: If the PUCAI increases by ≥20 points from the previous week the dose will be increased to the last dose at

which the patient had improvement in symptoms and maintained at that dose for one week. If the initial dose was <1.5 mg/kg/day (and <60 mg/day) and the patient maintains a PUCAI >34 the clinician may increase the oral prednisone or liquid equivalent prednisolone to 1.5 mg/kg/day (max 60 mg/day) for up to one week. Failure to respond at that point will prompt consideration of rescue therapy.

Subsequently, if the PUCAI decreases by ≥20 points from the increased PUCAI that necessitated an increase, the dose can then be lowered by the protocol above. If the PUCAI increases by <20 points from the previous week then the attending physician will have the discretion to leave the dose the same and re-assess in one week.

CS weaning from 12 weeks to 24 weeks: For those patients still receiving prednisone or liquid equivalent prednisolone at 12 weeks, further weaning will be at the discretion of the attending physician. Initiation of rescue therapy with IM or infliximab can occur at the discretion of the attending physician during weeks 12 to 24. Any patient who has failed to totally wean prednisone or liquid equivalent prednisolone by 24 weeks will be considered a treatment failure.

7.4. Definitions of CS Status

CS-free: Subjects will be considered to be CS-free if they are taking no CS at the assessment and have been CS-free for a minimum of 2 weeks at that point. Alternate day CSwill not be considered CS-free.

CS responsive: Clinical improvement and termination of CS within 12 weeks of initiating therapy, and remaining off CS for the remainder of the first year for a minimum of six months following initial tapering before requiring re-treatment with CS.

CS dependent: Required CS >12 weeks to control symptoms, or at least one additional course of CS starting <6 months after initial successful CS weaning

CS refractory: Unresponsive to oral CS within four weeks of starting therapy and required rescue therapy with CI or anti-TNFα therapy; or required rescue therapy with CI or anti-TNFα therapy because of failure to respond to intravenous CS, or required colectomy because of failure to respond to CS.

7.5. Criteria to Institute Rescue or Second Line Therapy

If there is a lack of sustained response/remission with use of mesalamine as a maintenance agent, or if there is an adverse reaction to mesalamine preventing its use as a maintenance agent, or if the patient is CS refractory or dependent then rescue or second line therapy should be instituted. The following will establish the need to institute rescue/second line therapy:

- The PUCAI continues to be ≥34 despite a minimum of 2-4 weeks of ≥1 mg/kg/day prednisone or liquid equivalent prednisolone
- Failure to wean prednisone or liquid equivalent prednisolone below 0.5 mg/kg/day by week 10 after commencing initial steroid therapy
- Continued activity (PUCAI >10) and need for CS at weeks 12-24
- Clinical relapse within 6 months of initial successful weaning of prednisone or liquid equivalent prednisolone

- Adverse reaction to mesalamine
- Hospitalized patient with severe colitis (PUCAI ≥65) and failure to respond to intravenous CS within 14 days, or at the discretion of the attending physician rescue therapy with a calcineurin inhibitor or infliximab is warranted because of continued refractory/fulminant disease. Specific guidelines will be provided as per recent consensus recommendations[12, 155].

The choice of rescue or second line therapy will be at the discretion of the attending physician. Below are guidelines for use of such therapies to establish a standard-of-care amongst investigative centers.

It is possible that rescue/second line medications will be given for reasons other than continued active ulcerative colitis. Examples would be concomitant autoimmune hepatitis associated with ulcerative colitis, or arthritis. In these cases the patient would be considered a failure with respect to the primary outcome at one year on an intent to treat basis ((ITT) but would continue to be followed.

Immunomodulator or biologic therapy: If a thiopurine is used then dosing of azathioprine (2.5-3 mg/kg/day) and 6-mercaptopurine (1-1.5 mg/kg/day) will be initiated assuming normal baseline thiopurine methyltransferase (TPMT) activity. If TPMT activity is intermediate the starting dose of azathioprine will be 1.25 mg/kg/day and the starting dose of 6-mercaptopurine will be 0.5 mg/kg/day. Subsequent measurement of 6-thioguanine metabolites will be at the discretion of the attending physician. Patients who require thiopurine therapy will be considered treatment failures with respect to the study's primary outcome. If the clinician chooses to use methotrexate as the initial or subsequent immunomodulatory therapy dosing will be at the discretion of that physician. It is suggested that all patients receiving IM will have regular monitoring of the laboratory tests listed in Table 7.3. The timing of monitoring noted below is will respect to the first day the IM is administered.

Table 7.3. Suggested laboratory tests to be monitored following institution of immunomodulator therapy

Lab Test	Baseline (Week 0)	Week 1	Week 2	Week 4	Week 8	Every 8-12 weeks
TPMT activity*	For thiopurine treated patients only					
Amylase/Lipase*	X		X	X	X	X
CBC, differential	X	X	X	X	X	X
AST/ALT	X		X	X	X	X
BUN/CR†	X		X	X	X	X
Drug levels (cyclosporine, tacrolimus) †		X	X	X		X

* For those on immunomodulator therapy

† For those on cyclosporine, tacrolimus

Calcineurin inhibitor (cyclosporine, tacrolimus) or anti-TNFa therapy: These therapies may also be instituted if in the judgment of the attending physician immediate rescue therapy is warranted. Additionally, anti-TNFα therapy can also be used for non-fulminant disease in lieu of IM for CS-dependency or CS-refractory disease. The dosage of these medications will be determined by the attending physician and recorded on the CRF. Patients who require cyclosporine, tacrolimus, or anti-TNFα will be considered treatment failures with respect to the study's primary outcome but will continue to be followed in the study for clinical outcomes as well as safety observations. It is suggested that patients receiving these medications will have regular monitoring of the laboratory tests listed in Table 7.3. Prior to anti-TNFα therapy all patients will be screened for tuberculosis with either a PPD (5 units) or QuantiFERON-Tb test, and a chest radiograph.

7.6. Concomitant therapy

Rectal therapy: Rectal therapy with either 5-ASA suppositories/enemas/foam or hydrocortisone suppositories/enemas will be allowed at the discretion of the physician for either severity cohort and will be recorded on the CRF.

Probiotics/Fish oil: Probiotic therapy or fish oil will not be given per protocol. The use of these preparations will be discouraged. If a patient is reported to be receiving either probiotics or fish oil that will be noted on the CRF but the patient will continue to be followed.

Antibiotics: If a patient is receiving an antibiotic for a presumed enteric infection at the time of diagnosis of ulcerative colitis, and investigation has shown no enteric infection, then the antibiotic will be stopped at the time the patient starts standardized therapy with either mesalamine or prednisone or liquid equivalent prednisolone. If a patient during the study receives an antibiotic for a gastrointestinal or extra-gastrointestinal illness the use of the antibiotic will be recorded on a CRF.

Other medications: Anti-diarrheal medications (loperamide, diphenoxylate HCl) will not be allowed for 48 hours prior to each determination of a PUCAI score. Their use at other times will be recorded on the CRF. Other preparations such as iron, multivitamins, vitamin D supplements, and calcium will be used at the discretion of the prescribing physician and recorded on the CRF. Patients will be discouraged from taking non-steroidal anti-inflammatory agents (NSAIDs). If taken their use will be recorded on a CRF. Patients will be prohibited from taking aspirin or other products containing, or metabolized to mesalamine (e.g., sulfasalazine, olsalazine, balsalazide). Vitamin D supplementation can be given at the discretion of the site investigator based on locally determined serum Vitamin D levels and customary practice. The use of supplemental Vitamin D and dose will be recorded on CRFs.

7.7. Intolerance to mesalamine

Intolerance to mesalamine: Severe intolerance to mesalamine (paradoxical disease worsening, pancreatitis, hepatitis, or other significant side-effects) that preclude its use as a maintenance agent will constitute a treatment failure with respect to the primary study outcome and will prompt the use of IM or anti-TNFα for maintenance therapy. These patients will continue to be followed and have clinical outcomes recorded. The reasons for mesalamine intolerance will be recorded in the CRF. It is anticipated that some patients may develop more

mild signs of intolerance such as headache or nausea. In that case an attempt will be made to initially lower the mesalamine dose by 50% and then if now tolerated the site will gradually increase the dose back to the standardized dose if possible. If there is loss of efficacy of the reduced mesalamine dose, and the patient cannot tolerate an increased dose, this will be considered failure and will prompt the use of other second line agents.

8. ADVERSE EVENTS

PROTECT involves the study of a treatment protocol for ulcerative colitis. The treatment plan was arrived at by consensus among the investigators and is considered standard-of-care rather than investigative. The study does not involve comparisons between treatments nor is there any use of medications not yet licensed for use in humans. The investigators have obtained an IND (111863) for Pentasa (mesalamine) use in the pediatric population.

During telephone contacts and follow up visits, study participants will be asked to report on any hospital or other medical visits since their last study contact, and on any possible adverse reactions. All potential serious adverse events or reported side effects will be recorded as study data.

8.1. Definitions of Adverse Event and Serious Adverse Event

An **adverse** event is any untoward or dangerous reaction to a study intervention.

A **serious adverse event** is an adverse event that:

- Results in the participant's death;
- Is life-threatening;
- Results in hospitalization (initial or prolonged);
- Results in significant, persistent, or permanent change, impairment, damage, disruption, or disability in the participant's body function/structure, physical activities or quality of life;
- Results in a congenital anomaly; or
- Requires intervention to prevent permanent impairment or damage.

Non-serious adverse events are all adverse events that do not meet the above criteria for "serious."

8.2. Reporting of Serious and Other Adverse Events

All serious adverse events will be reported to the DCC within 24 hours of the clinical site personnel becoming aware of the occurrence of the event. Clinical sites are responsible for reporting adverse events (serious or non-serious) to their local IRB in accordance with the local IRB requirements. The medical director at the site will determine the proper response per the research protocol, i.e., changing therapy or initiating new therapy.

The DCC will report serious adverse events to the study safety officer and the independent Data Safety Monitoring Board (DSMB) according to the guidelines and schedule established by that group. All adverse events and safety data will also be reported to the DSMB in regularly scheduled reports.

Because the study will be conducted under an IND, the DCC will also be responsible for reporting adverse events and serious adverse to the FDA. Anticipated adverse events based upon previous experience in adult patients occurring with a frequency of ≥1% include diarrhea, headache, nausea, abdominal pain, dyspepsia, vomiting, and rash.

9. CENTRAL LABORATORIES

9.1. Biospecimen Collection and Shipping

As noted in Table 6.5, biospecimens will be collected at several specified time points in the study. Standard of care laboratory testing including complete blood count, serum chemistries, serum aminotransferases, renal function studies, erythrocyte sedimentation rate, C-reactive protein, and urinalysis will be performed at the local clinical laboratories of each participating institution and will be monitored as part of routine clinical care. TPMT testing will be sent to a reference laboratory determined by the clinical site. Results will be recorded on CRFs. Harmonization of reference units will be made so data will be comparable between sites. Biospecimens for research purposes will be shipped to the PROTECT biospecimen repository at Emory University School of Medicine.

9.2. Biospecimen Analysis

The biospecimen repository will send biospecimens to various facilities for analysis as noted in the list below.

Cincinnati Children's Hospital and Medical Center: Serum vitamin D ; colon RNA-seq; fecal calprotectin.

Emory University: Genotyping.

Connecticut Children's Medical Center: Fecal osteoprotegerin (OPG).

Cedars-Sinai: IBD serology.

Broad Institute: Microbiome.

10. DATA MANAGEMENT

10.1. Data Management System

A web-based data management system (DMS) will be used for this study. The data management system will provide all of the capabilities required for research data management, including: data transfer, data entry, data validation, database updating, database closure, data retrieval, data inventory, security and confidentiality, and archiving, and in addition will support eligibility determination. Each clinical site will be responsible for entering the data it collects. The clinical site staff will use the DMS to enter screening and eligibility data and run an algorithm to check eligibility. Follow-up data will also be entered into the DMS at the clinical sites.

The server and main database reside at the DCC at the Collaborative Studies Coordinating Center (CSCC) at the University of North Carolina at Chapel Hill.

10.2. Data Collection, Entry, Editing and Reporting

Data are collected at clinic visits and by telephone interviews. Direct data entry, where data initially are entered on the screen without having completed a paper form first, will be available at each center. Direct data entry eliminates the time-consuming and error prone process of keying from paper forms. Paper versions of each data collection instrument will be available as backup in situations in which the computer systems are inaccessible for any reason. In addition, if there are forms that are routinely collected on paper for convenience or another reason, then the data on these forms will subsequently be keyed at the clinical sites using the web-based DMS.

The data entry system will display data entry screens that closely resemble the paper data collection forms. The system will be menu driven, with context-sensitive help available at any time. Each data field will be edited during entry.

The DMS will include the ability for each center to generate locally a variety of summary reports concerning the data completeness, outstanding questionable values, etc. This capability is valuable in permitting study coordinators to monitor the quality of their site's performance. This facilitates timely identification and resolution of problems in data collection and processing.

10.3. Central Laboratory Management System

Laboratories typically have their own data management systems. The central laboratories will prepare data files from their local data management systems in a standardized format and transfer these to the DCC on a regular schedule. Upon receipt at the DCC, these data files will be processed for incorporation into the study's consolidated database.

10.4. Confidentiality and Security

The PROTECT clinical database is housed in a secure, climate controlled server facility at UNC. The CSCC has in place an IT Security Plan as required by NIH contracts and grants. The plan documents standard operating procedures required to secure the CSCC network and databases, including management, operational and technical controls. As part of the plan, the

principle of least access privilege for study files is implemented. Included in the plan are a risk assessment, a system continuity plan, and a disaster recovery plan.

Data confidentiality and security measures are applied at all levels of PROTECT data acquisition, transfer and storage, and applied to all study agencies, including the clinical centers, the DCC, central laboratories, and any reading center that may be engaged for the study. The PROTECT DMS meets exacting data management standards of confidentiality, as well as HIPAA requirements. Beyond the password-controlled access to the study equipment and the DMS, data collected at the clinical centers are encrypted by the system and can only be decrypted for display on-screen by authorized study personnel. Personal identifiers are collected on separate CRFs as an additional safeguard against linking of identifiers and medical information. The DCC is responsive to data confidentiality requirements originating from providers of medical care or IRBs, as needed to enable the work of the clinical centers. It is a goal of the PROTECT study to collect all data electronically however, should paper data collection forms be used they will be retained at secure locations at the clinical centers until the Steering Committee acts on recommendations from the DCC to dispose of such records (e.g., after incremental data closure). The secure storage and disposition of hard copy records at clinical centers will follow institutional procedures at each site.

As standard practice, output mailed or otherwise sent to a clinical center identifies participants only by ID number. At the CSCC, printed material containing confidential information is discarded through supervised loading, transportation, and storage using a chain of custody control process, until the material can be recycled into paper pulp.

Any personally identifying information such as dates of medical procedures that are entered into the central DMS will be stored on a secure server at UNC following the procedures described above.

10.5. Records Retention

10.5.1. Data Coordinating Center

The DCC will comply with all local and federal regulations in maintaining study related documentation. This documentation includes financial records, supporting documents, and all records related to the award. NIH policy states that these must be kept for at least three years after study closure[156].

10.5.2. Central Laboratories and Biospecimen Repositories

The biospecimens stored at central facilities remain the property of the PROTECT Study. The samples are available only to approved investigators for approved uses. For any use beyond what is stated in this protocol, investigators must submit an application to the Ancillary Studies Committee. Any unused biospecimens will become the property of the NIH at a time to be agreed between the PROTECT Steering Committee and the NIDDK Project Office.

Samples sent to the laboratories will be identified only by a laboratory sample ID label, distinct even from the unique study ID assigned to each participant. In particular, no PHI (Protected Health Information) identifiers will be sent to the laboratory. The DCC will maintain the link between the participant ID and the laboratory sample ID and this link will not be shared outside the DCC.

Regarding the destruction of samples, it is PROTECT Study policy that participants retain their rights to have their samples removed from the laboratory inventories at any time and have no further analytical disbursements performed. The withdrawal request must be made to the originating PI. This individual PI will transmit a request to the DCC, which will identify the link between the participant ID and the laboratory sample ID and generate a removal/destruction request for all samples and records associated with a specific laboratory sample ID. Samples already released to approved requesting investigators cannot be returned or destroyed. In addition data generated prior to a participant's request for sample removal will not be destroyed.

The only other time biospecimen sample will be destroyed is at the request of a site PI when a participant turns out to be ineligible, in accordance with the IRB documentation. At this point both the sample and any data associated with it are destroyed in the same manner as described above.

10.5.3. Clinical Sites

The secure storage and disposition of hard copy records at clinical centers will comply with local institutional procedures as well as federal regulations.

11. QUALITY ASSURANCE

11.1. Training and Certification

All staff involved with data collection will be required to have appropriate training and certifications. Each clinical site will have a study coordinator or project manager who has received formal training from the DCC about the PROTECT protocol and procedures. This individual may then train other local staff in study procedures. A copy of the PROTECT Manual of Procedures will be given to all investigative sites and will be used for training and standardization of procedures. This manual will be submitted to the FDA and investigative sites prior to initiation of this study.

11.2. Data Reporting for Quality Assurance

Monthly data reports of study status and data quality are prepared by the DCC and posted on the secure study website. Data will be summarized overall and by clinical site. These reports include information such as the number of patients screened, number enrolled, percent of follow-up contacts completed, and the number and percent of missing forms. In addition, the DCC will generate site-specific reports for data quality, such as missing and overdue forms, missing or suspicious data items, outstanding data queries, etc., and facilitate the timely review and resolution of data quality issues within the study.

11.3. Site Monitoring Visits

The DCC will conduct periodic monitoring visits to each participating clinical site, and central laboratory. DCC monitoring visits will be made annually to each site. In addition to evaluating the quality with which the study is being conducted at individual sites, monitors will assess specific implementation methods, compare implementation strategies across sites, and make as well as receive suggestions for improving study performance. Monitors will review research and medical records of PROTECT participants for accuracy of case report forms. In addition, if enrollment at a site falls below a certain level or other problems with study conduct arise, a more diverse site visit team may be initiated. This larger site visit team would include personnel designated by the Executive Committee, such as a team consisting of a clinician from a highly productive center, and representatives from the NIDDK Project Office and DCC.

11.4. Replicate Measures Program

Some procedures or data collection may be repeated for quality assurance purposes. When this involves additional participant burden, informed consent will be obtained. A subset of participants will be asked to provide additional biospecimens for blinded laboratory analysis and quality assurance comparison with the study values. These additional biospecimens will be processed, shipped, stored and assayed or examined in exactly the same way as the regular study samples. The "blinding" involves labeling the samples in such a way that laboratory personnel are not aware which samples are regular study samples and which are quality control replicates. The samples which may potentially be most affected by variability in shipping and storage processes will be the blood samples used to prepare plasma. In order to test the reliability of our sample shipping, processing, and storage processes, duplicate blood samples will be collected into BD P100 tubes from 40 subjects. The BD P100 tubes will be shipped from the study sites to the biorepository on different days, and processed and stored separately.

Plasma aliquots from these duplicate samples will be used to measure 25OHD at the CCHMC site, and results will be tested for variance. The DCC has standard programs for analyzing results from these blinded replicate pairs.

12. STATISTICAL ANALYSIS

12.1. Analysis of the Primary Endpoint

The primary endpoint is SFR at 52 weeks. To define rates of SFR at week 52 on mesalamine the analysis will be restricted to patients who are able to initiate standardized induction therapy and do not require rescue therapy within 4 weeks. The primary hypothesis is that the proportion of patients in SFR at 52 weeks will be higher among those who have PUCAI <10 at week 4. Because patients may receive rescue therapy at any time and some may be lost to follow-up, we will analyze the primary endpoint using time-to-event (survival analysis) methods, with the event being initiation of corticosteroids or rescue therapy. Patients will be censored at the time of loss to follow-up or the end of the period of interest, if still in SFR remission at that time. The initial analysis will use the log-rank test to compare time-to-event between those with PUCAI < 10 at week 4 and those with PUCAI ≥ 10, first using all patients who did not require rescue therapy within 4 weeks and then stratified by the patients' disease severity at diagnosis (Mild (PUCAI < 34) vs. Moderate/Severe (PUCAI ≥ 34)) and mean adherence to prescribed mesalamine therapy (≥80% or <80%). Additional analyses will use proportional hazards models to adjust for covariates including disease severity at diagnosis (Mild vs. Moderate/Severe). We will use the proportional hazards models to estimate the proportion (and associated 95% confidence interval) in each group who are in SFR at remission at 52 weeks.

We will not consider the effect of different induction therapies explicitly as the induction therapies will be prescribed based upon the disease severity at diagnosis and their effects may be confounded with the effects of the disease severity. Calculation of adherence is described below.

12.2. Analysis of the Secondary Endpoints

Some of the secondary endpoints are the same as the primary endpoint except that they are over different time intervals (12, 26 or 104 weeks). For these and for time to colectomy (or censoring) we will use the same approach as for the primary endpoint.

Other endpoints are dichotomous and assessed at a specified time rather than occurring at arbitrary times (for example, PUCAI < 10 at 4 weeks). For these and as a sensitivity analysis for the primary endpoint, we will calculate the proportions at the specified time points among those with PUCAI < 10 at week 4 and among those with PUCAI ≥ 10 at week 4, first using all patients who did not require rescue therapy within 4 weeks and then stratified by the patients' disease severity at diagnosis (Mild (PUCAI < 34) vs. Moderate/Severe (PUCAI≥ 34)) and mean adherence to prescribed mesalamine therapy (≥80% or <80%). We will use a two-sample test of proportions to compare the pooled (or unadjusted) proportions. We will then use a Cochran-Mantel-Haenszel (CMH) test to compare the stratified proportions (adjusted for the initial disease severity and adherence to prescribed mesalamine therapy). The proportion in SFR, whether unadjusted or adjusted, may vary across clinical centers and we will use a generalized linear mixed model (GLMM) to accommodate the center-to-center variability in comparing the proportions.

12.3. Prediction Models

We will use the study sample as an index set and logistic regression to build a prediction model for the probability of the SFR outcome at week 52. We will first include the following variables to test their hypothesized predictive powers, adjusted for adherence to prescribed 5-ASA medication: (1) age > 12, (2) female gender, (3) mild clinical disease activity at diagnosis (PUCAI <34), (4) serum 25-OH vitamin D > 15 ng/mL at diagnosis. 6) serum GM-CSF Ab < 0.6 mcg/mL at diagnosis, 7) Mayo score of 1 at diagnosis (mild endoscopic severity), 8) fecal calprotectin at diagnosis, and 9) fecal OPG at diagnosis. We will then include additional clinical, genetic/genomic, and immune variables via a forward stepwise variable selection procedure. This will include testing the ability of candidate genetic variants associated with week 52 SFR identified by our genotyping array results to improve the predictive power of the model. On a subset of the patients where data are available for the following factors: (10) PUCAI < 10 at week 4, 11) change in fecal calprotectin between entry and week 4, (12) fecal calprotectin ≤ 150 mcg/gm at week 4 or 12, 13) change in fecal OPG between entry and week 4, and/or (14) fecal OPG ≤ 50 pmol/L at week 4 or 12, we will test their predictive powers in addition to the variables (1)-(9) above. In each case we will use the area under the (receiver operating characteristic) curve (AUC) to summarize the operating characteristics of the prediction model, and determine sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). We will use bootstrap method to calculate the standard errors of each statistics. To check the fit of the logistic regression model, we will use the Hosmer-Lemeshow goodness of fit test. We will also generate a plot of the absolute risk for week 52 SFR as a function of the risk score.

12.4. Adherence Data

We will calculate the adherence percentage at each assessment time point using the Medication Event Monitoring System (MEMS®) measured electronic data. Initially adherence will be dichotomized into high versus low, defined as above or below 80% of the prescribed dose. In secondary analyses we will investigate using other cut-points to categorize adherence, as the 80% cut-point which is used by convention has not been empirically tested for association with clinical outcomes in this setting. Missing electronically measured adherence data will be imputed based on pill count data by standard imputation methods [157] and imputed values will be used in the analysis.

As well as using adherence as a predictor of other outcomes, we will also analyze adherence as an outcome. We will use data collected using the BASC-2 as a potential predictor of adherence, as well as of week 52 SFR. We also will investigate whether there are any systematic longitudinal patterns (e.g., constant “high” or “low” adherence, or tapering off adherence). If such patterns exist, we will conduct association analysis to see whether the systematic patterns are significantly associated with the week 52 SFR rate. After classifying patients into the systematic patterns we will use indicators of the patters in a logistic regression model with week 52 SFR as dependent variable.

12.5. Endoscopic Response and Remission

Endoscopic response (Mayo endoscopy sub-score reduced by ≥1 and being 0 or 1) and remission (Mayo score 0) at week 52 are dichotomous secondary outcomes However, these outcomes may require a more sophisticated analysis than the other dichotomous outcomes.

Because sigmoidoscopy at week 52 is not standard of care the costs of the procedure will have to be borne by the study. Patients who have already failed the primary outcome prior to 52 weeks (i.e., have received rescue therapy, are still on corticosteroids, or have had colectomy) will not be eligible for study sigmoidoscopy at week 52. Only those subjects who are in SFR will be eligible for week 52 study sigmoidoscopy. The way in which these patients are selected and factors influencing whether they consent to the procedure, means that the mechanism determining who has missing data may be non-ignorable. The missing mechanism, if non-ignorable and unaddressed, may introduce bias into the statistical analysis. We will perform two analyses, one using complete cases only by assuming the missingness is non-informative and the other using both complete and missing cases by the estimation maximization (EM) method. If the two analyses differ little, it will indicate the missingness is ignorable. We will assume the missing data depend only on a group of clinical variables.

12.6 Genotyping Method, Quality Assurance, and Analysis

Genotyping: We will use the genotyping core established by Dr. Kugathasan at the Emory University Center for Medical Genomics (CMG) for the CCFA-sponsored CD RISK study. We will genotype 475 cases using the ImmunoChip (Illumina Human Immuno DNA Analysis BeadChip). The ImmunoChip has ~196,000 SNPs, including ~6400 that cover the extended MHC region, ~5000 ancestry informative markers (AIMS), and ~760 SNPs across each of ~180 loci that have been associated in 12 immune mediated diseases including UC with statistical support that reaches genome wide significance. We have chosen to use the ImmunoChip instead of a custom made iPLEX SNP array for variety of reasons; 1) very cost effective for testing a large number of SNPs, 2) no need for genotyping controls as well over 10,000 disease free controls with ImmunoChip genotyping data are available for analysis, 3) genotyping of common and rare variants, 4) fine mapping of all known IBD loci including UC, and 5) inclusion of AIMs allows us to perform accurate population stratification between cases and controls. Individual SNPs with 5-ASA pharmacogenomic significance including genes such as NAT1, NAT2, ABCC1(MRP1), ABCC2(MRP2), ABCC3(MRP3), ABCC4(MRP4), UGT1A6, UGT1A9, UGT1A7, UGT1A1 ABCB1(MDR1), ABCG2(BCRP), SLCO1A1(OATP1A1), SLCO1A3(OATP1A3), GSTM1, GSTT1, GSTP1, GSTA1, SULT1A1, SULT1A2, SULT1A3, Nrf2 that are not covered by the ImmunoChip will be performed by Taqman genotyping. SNPs encoding these genes will be selected using haploview.

Quality control: Rigorous QC procedures will be applied to genotyping and analysis. Genotyping will be performed using the Illumina Infinium assay protocol. The florescent activity of the chip is detected using the Illumina HiScanSQ instrument. Data captured by the reader are normalized and transferred into the software package BeadStudio (Illumina) to convert the fluorescent information into SNP genotypes. Replicate genotyping across experiments will be performed to ensure concordance. Genotypic data will be used only from samples with call rates >95% and SNPs with a call frequency >95%. SNPs out of HWE in controls with $p < 0.001$ will be discarded. Cases and controls will be randomly distributed on the plates for genotyping. We will also genotype individuals from the Coriell HapMap collection with known genotypes as another QC check for our genotyping.

Data analysis: For our analysis, cases will be subjects who achieve week 52 SFR, and controls will be subjects who do not achieve week 52 SFR. Population stratification is always a

concern in association studies. We will address the effects of population stratification. We will only include samples of individuals of European ancestry in the initial analysis as the number of non-European samples is expected to be underpowered to detect any differences, and we will further ensure homogeneity by using principal component analysis (PCA) to identify and potentially eliminate individuals that disproportionally contribute to stratification. We will evaluate data for missing data proportions between cases and controls, as well as departures from Hardy-Weinberg equilibrium (HWE). We will estimate marker-marker LD and test for allelic and genotypic cases-control associations. We will calculate OR, 95% CIs, and probabilities. We will also evaluate haplotypes in the fine-mapping regions of the ImmunoChip for associations. EM-algorithm-based haplotype frequencies will be calculated and tested for differences between cases and controls. We will use likelihood ratio statistics and permutation based estimation of p values, using PLINK.

Anticipated results and potential pitfalls: The ImmunoChip has hundreds of variants at loci associated with UC and other autoimmune disorders at genome-wide significance. However, because our sample size is modest by GWAS standards, we will only be powered to detect large effect loci (ORs > 1.3 if we correct for the number of independent regions, and > 2.0 if we correct for all ~196,000 markers) for association with week 52 SFR. Since the RISK samples would have already undergone ImmunoChip analysis, we will use 400 RISK study UC subjects as a replication cohort or cross validation with the PROTECT cohort for the outcome of week 52 SFR. However, UC patients in RISK will not have received standardized therapy, or adherence monitoring. If we detect such an association, due to the dense coverage of each of these loci in the ImmunoChip, we expect to have a relatively finely mapped region containing that association. Based upon the demographics of pediatric UC in North America, we anticipate that 91% of the cohort will be white, 8% African American, and 1% other race/ethnicity. Even with RISK and PROTECT in combination, we are underpowered to investigate subjects of non-European ancestry for association. However, we will test individual SNPs and haplotypes for association in all ethnicities if statistically significant association is found even with small numbers.

12.7. Sample Size and Statistical Power

As noted above, the primary efficacy endpoint is being in SFR with mesalamine only as maintenance therapy at week 52. Our proposed sample size of n = 475 is based on observations from a cohort of 353 pediatric UC patients currently enrolled in the Pediatric IBD Collaborative Research Group Registry (unpublished data). In this Registry clinical remission was defined as a Physician Global Assessment (PGA) of inactive. The PUCAI measurement of disease activity has recently been added to PGA for disease activity assessment.

In our Registry data we observed that 20% of the UC patients required rescue therapy with surgery or medication escalation prior to week 4 post-diagnosis. Week 52 SFR was 59% in those in remission (PGA inactive) at week 4, compared to 29% in those with PGA active at week 4 (OR = 3.3, 95% confidence interval (1.9, 5.6), $p<0.0001$), among the remaining 80% who had not required rescue therapy. We conservatively anticipate two-fold higher odds of week 52 SFR rate in pediatric UC patients who achieve remission (as defined by PUCAI < 10) at week 4 after initiation of therapy. We also assume conservatively that 10% will drop out of the study. For the analyses we will initially assume that patients who drop out will not be in remission at 52 weeks. (In sensitivity analyses we will investigate how this assumption

influences results.) The proposed sample of n=475 will provide >80% power to detect a two-fold higher odds of week 52 SFR, accounting for the 20% that will require rescue therapy prior to week 4, at a significance level of 0.05. The power calculation was conducted using POWER V3.0. The power calculations are based on proportions at 52 weeks rather than on time-to-event analyses because of the lack of adequate data on which to base time-to-event models.

We consider it to be unlikely that substantially more than 20% of patients will require rescue therapy by week 4. However, if this does occur it will reduce study power. For each DSMB report while recruitment is ongoing, the DCC statisticians will calculate the percentage requiring rescue therapy by week 4, for consideration of the potential effect on study power. This will be done without conducting a test of the primary outcome and so does not constitute an interim analysis for efficacy or futility.

Power calculations were based on a planned sample size of n=430 participants. A sample size of 475 will facilitate enrichment of complete biospecimen ascertainment at critical study visits.

Table 12.1. Anticipated power assuming 30% SFR percentage at week 52 in those without the predictor, adjusted to 27% by counting drop-outs as not being in remission (n=430 participants)

Prevalence of Predictor	OR = 2	OR = 2.5
40%	85%	98%
30%	80%	96%
20%	70%	90%

To identify other clinical, genetic, and immune predictors of SFR (PUCAI<10) at week 52, we will utilize the pre-specified variables summarized in Table 12.2 and additional variables as identified in our genetic, genomic, immune, and serologic studies in a multivariate logistic regression. If the prevalence of potential dichotomous predictors is in the range of 20%-40% with OR ≥ 2, we anticipate power of at least 70% for each of the predictors (see Table 12.1). Utilizing the rule of thumb of 10 events per degree of freedom for an adequately powered and not overly fitted logistic model, our sample should allow the inclusion of 10 variables.

Table 12.2. Variables to predict SFR rate at week 52

Variable	**OR (95% CI)**	**p-value**
Age ≥ 12	1.02 (0.60, 1.74)	0.94
Female gender	0.78 (0.46, 1.32)	0.35
White race	1.04 (0.50, 2.19)	0.91
Pan-colitis involvement	0.91 (0.47, 1.80)	0.80
Mild colitis at diagnosis	1.65 (1.03, 2.64)	0.04
Week 4 PGA inactive	3.29 (1.93, 5.61)	<0.0001

OR: odds ratio for week 52 SFR from preliminary studies.

13. DATA AND SAFETY MONITORING PLAN

13.1. Monitoring Data and Safety

In any study involving humans, the safety of the participants is paramount. The treatment protocol in PROTECT is regarded as standard of care by the investigators rather than being experimental. There is minimal additional risk specific to study procedures. The primary study-specific procedures are biospecimen collection and, in a subset of patients, a sigmoidoscopy at week 52. Nevertheless, information on all adverse events will be collected and reported appropriately to IRBs, a study safety officer, the Data and Safety Monitoring Board (DSMB), and the FDA. Details about what constitutes an adverse event and reporting requirements are provided in section 8 of this protocol. Risks and benefits to participants are addressed in section 6.2.4.

It is unethical to conduct a study with a sample size that is too small to provide adequate power to test the primary hypothesis. In addition, substantial missing data, whether by loss to follow-up, missed visits, or inadequate procedures, may bias analyses. Thus it is important to monitor enrollment in order to keep the study on track to meet its enrollment target and also to monitor the completeness and quality of the data collected. The DCC will provide the Steering Committee with monthly reports containing data on enrollment, completion of study visits, completion of data collection and quality of data.

13.2 Study Safety Officer

Sites are asked to notify the DCC of any serious adverse events within 24 hours of becoming aware of the event. Once the DCC is notified, the PROTECT independent study safety officer will be informed of the serious adverse event within one business day. The study safety officer will review the site investigator's assessment of the relationship of the study drug to the serious adverse event, the expectedness of the event, and to make sure that the narrative of the event is well and completely documented for the DSMB, NIDDK, and FDA.

13.3. Data and Safety Monitoring Board (DSMB)

13.3.1. Role and Composition of the Data and Safety Monitoring Board

The DSMB will be appointed by and report to the NIDDK. The DSMB is comprised of independent scientists who monitor the progress of the study and the safety of the participants. The attention of the DSMB will focus on patient accrual, appropriate follow-up, compliance, data acquisition, undue complications, and whether the study as it is currently being conducted will be able to answer the hypotheses it addresses. The responsibility for participant safety is particularly important. At each meeting while the study is in the field, the Board will review all adverse events.

The membership and frequency of meeting are at the discretion of NIDDK. The DSMB is likely to consist of at least 5 members including a biostatistician and 3 clinical investigators. It is anticipated that the Board will meet twice a year, with one of these being an in-person meeting and the other a teleconference.

13.3.2. DSMB Reports

The DSMB reports will be prepared twice a year (or as specified by the DSMB). Although the DSMB will determine the format of the report, we anticipate that each report will consist of six sections: 1) recruitment, 2) treatment, 3) adverse events, 4) patient adherence, 5) data quality, and 6) sub-studies. The recruitment section will present overall recruitment, as well as by UC severity at baseline, and for other subgroups of interest (e.g., by gender). The treatment section will contain information about the treatment status of patients. The section on adverse events will provide a summary of all adverse events and serious adverse events (SAE) along with details about each SAE. Patient adherence data will display adherence patterns over time and compare adherence between groups of patients, such as by baseline UC severity. The quality control sections will include summaries of the quality control data collected by the DCC to monitor and correct operational data collection. Any sub-studies will be monitored to ensure that they do not adversely affect recruitment or adherence.

Because this study is not comparing treatments, there will not be any interim analyses for efficacy or futility.

The DCC will provide data management and statistical computing to support the monitoring of the study. Recommendations concerning the continuation or cessation of the trial will be made by the DSMB to the NIDDK.

14. STUDY AND COMMITTEE ORGANIZATION

14.1. Participating Entities

14.1.1. Clinical Treatment Centers

Clinical treatment centers will be selected from the roster of those currently participating in the Crohn's and Colitis Foundation of America (CCFA) sponsored study entitled " Risk Stratification and Identification of Immunogenetic and Microbial Markers of Rapid Disease Progression in Children with Crohn's Disease" (www.prokiids.com). This is a 4 year inception cohort study of 1200 pediatric patients with Crohn's disease which will define clinical, genetic, genomic, and serologic predictors for aggressive disease behavior. Successful clinical monitoring and biospecimen procurement have been demonstrated in the RISK study by all study sites who will participate in The PROTECT Study. Sites that have not participated in RISK will be eligible if they see an adequate number of new patients with ulcerative colitis each year and have a dedicated research coordinator and principal investigator.

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14.1.2. Clinical Coordinating Center

Location	**Principal Investigator**
Connecticut Children's Medical Center	Jeffrey Hyams, MD

14.1.3. Data Coordinating Center

Location	**Principal Investigator**
University of North Carolina at Chapel Hill	Sonia Davis DrPH

14.1.4. Central Laboratories and Biospecimen Repositories

Location	**Principal Investigator**
Emory University (biorepository)	Subra Kugathasan, MD
Cincinnati Children's Hospital and Medical Center	Lee Denson, MD
Cedars Sinai Medical Center	Shervin Rabizadeh, MD
UCONN Health Center	Francisco Sylvester, MD

14.1.5. National Institutes of Health

Institute	**Project Officers**
NIDDK	Frank Hamilton, MD, MPH
	Jose Serrano, MD, PhD
	Dana Andersen, MD

14.2. Committees

14.2.1. InvestigatorsCommittee

The Investigators Committee consists of the Principal Investigators of each of the clinical sites and central agencies and a representative from the NIDDK. The committee is chaired by Dr. Jeffrey Hyams. Dr. Lee "Ted" Denson is the Co-Chair and will serve as Chair if Dr. Hyams is unable to fulfill his role. Each of the clinical centers has one vote on the Investigators Committee. The representatives from the central agencies and NIDDK are ex-officio without vote.

The Investigators Committee meetings bring together investigators and clinical coordinators from the various participating centers for discussion regarding implementation of the protocol, study logistics, progress of the trial, possible changes in the protocol or methodology, new developments in the field, revitalization of interest, and other matters of concern to participants in the study. These meetings may also provide an opportunity for staff training and education. The Investigators Committee will establish subcommittees (see below) to develop and monitor aspects of the study, reporting recommendations to the Executive Committee for approval.

14.2.2. Executive Committee

The Executive Committee provides clinical and scientific direction for the study at the operational level. This committee consists of the investigators who took the lead in writing the application for funding of the cooperative agreement, namely Drs. Hyams, Denson, Walters and Davis, and the NIDDK Project Officer. Additional members of the Executive Committee will be Dr. Subra Kugathasan who will serve as Director of the central laboratory at Emory, and two site Principal Investigators who will serve for a one year term. Criteria for designation of these latter two members of the Executive Committee will be determined by the Investigators Committee.

The major responsibilities of the Executive Committee are reviewing overall progress of the study with particular emphasis on programmatic issues, including operational, budgetary, safety, compliance, and quality issues. In addition to formulating the Investigators Committee agendas, this Executive Committee will provide planning and organization for DSMB meetings, including review of preliminary, non-confidential data in preparation for these meetings. The Executive Committee in conjunction with the Publication Committee will oversee all aspects of the execution and publications of the study. The Executive Committee will meet as necessary with regular conference calls throughout the study, including the period for final analysis and writing activities that follow the conclusion of patient follow-up.

14.2.3. Ancillary Studies Committee

It is anticipated that both intramural and extramural investigators will wish to capitalize on the potential for collaborative ancillary investigations afforded by the implementation of the PROTECT study. The Ancillary Studies Committee will formally review and recommend approval or disapproval of all proposed ancillary studies, considering both their impact on the conduct of the PROTECT study, and their scientific merit.

14.2.4. Publications Committee

The Publications Committee will formulate publication policy for this collaborative research and review all abstracts, papers and scientific presentations which utilize study data. The Publications Committee will be responsible for identifying topics for publication as well as making writing group assignments. The committee will review and recommend approval or disapproval of all scientific abstracts and papers or presentations using unpublished study data, as well as every paper using published data that purports to represent official study views or policy. Another major responsibility of the Publications Committee is in the development of plans for the dissemination of study findings and incorporation of the findings into medical care policy. This will involve not only reports in medical journals but consideration of continuing education courses, conferences and seminars and special efforts such as press conferences, editorials, physician newsletters and presentations at local medical association meetings.

14.2.5. Study Coordinators Committee

This committee consists of the study coordinators from each of the clinical sites and central agencies. The committee will have regular conference calls to share information about best practices. A primary function of the committee is to facilitate communication among the sites and hence to ensure that study procedures are being applied uniformly at all sites.

14.2.6. Quality Control Committee

The Quality Control Committee will be responsible for ensuring high quality data by monitoring clinic, laboratory and other central agency performance and initiating corrective action when needed. Quality control reports provided by the DCC will be reviewed. A system for sending blinded replicate samples to the central laboratories will be developed by this committee and implemented by the DCC, with the results monitored by this committee.

14.2.7. Outcome Adjudication Committee

The Outcome Adjudication Committee will oversee questions concerning study outcomes as well as any concerns on patient eligibility for inclusion in the study and for consideration of that patient's eligibility to be part of the final study outcome.

14.2.8. Data and Safety Monitoring Board (DSMB)

The composition and roles of the Data and Safety Monitoring Board are described in section 13.

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APPENDIX A – Pentasa Package Insert

PENTASA®
(mesalamine)
Controlled-Release Capsules 250 mg and 500 mg
Prescribing Information as of June 2008 Rx only

DESCRIPTION

PENTASA (mesalamine) for oral administration is a controlled-release formulation of mesalamine, an amino-salicylate anti-inflammatory agent for gastrointestinal use. Chemically, mesalamine is 5-amino-2-hydroxybenzoic acid. It has a molecular weight of 153.14.

Each 500 mg capsule contains 500 mg of mesalamine. It also contains the following inactive ingredients: acetylated monoglyceride, castor oil, colloidal silicon dioxide, ethylcellulose, hydroxypropyl methylcellulose, starch, stearic acid, sugar, talc, and white wax. The capsule shell contains FD&C Blue #1, gelatin, titanium dioxide, and other ingredients.

CLINICAL PHARMACOLOGY

Sulfasalazine is split by bacterial action in the colon into sulfapyridine (SP) and mesalamine (5-ASA). It is thought that the mesalamine component is therapeutically active in ulcerative colitis. The usual oral dose of sulfasalazine for active ulcerative colitis in adults is 2 to 4 g per day in divided doses. Four grams of sulfasalazine provide 1.6 g of free mesalamine to the colon.

The mechanism of action of mesalamine (and sulfasalazine) is unknown, but appears to be topical rather than systemic. Mucosal production of arachidonic acid (AA) metabolites, both through the cyclooxygenase pathways, ie, prostanoids, and through the lipoxygenase pathways, ie, leukotrienes (LTs) and hydroxyeicosatetraenoic acids (HETEs), is increased in patients with chronic inflammatory bowel disease, and it is possible that mesalamine diminishes inflammation by blocking cyclooxygenase and inhibiting prostaglandin (PG) production in the colon.

Human Pharmacokinetics and Metabolism

Absorption. PENTASA is an ethylcellulose-coated, controlled-release formulation of mesalamine designed to release therapeutic quantities of mesalamine throughout the gastrointestinal tract. Based on urinary excretion data, 20% to 30% of the mesalamine in PENTASA is absorbed. In contrast, when mesalamine is administered orally as an unformulated 1-g aqueous suspension, mesalamine is approximately 80% absorbed.

Plasma mesalamine concentration peaked at approximately 1 mcg/mL 3 hours following a 1-g PENTASA dose and declined in a biphasic manner. The literature describes a mean terminal half-life of 42 minutes for mesalamine following intravenous administration. Because of the continuous release and absorption of mesalamine from PENTASA throughout the gastrointestinal tract, the true elimination half-life cannot be determined after oral administration. N-acetylmesalamine, the major metabolite of mesalamine, peaked at approximately 3 hours at 1.8 mcg/mL, and its concentration followed a biphasic decline. Pharmacological activities of N-acetylmesalamine are unknown, and other metabolites have not been identified.

Oral mesalamine pharmacokinetics were nonlinear when PENTASA capsules were dosed from 250 mg to 1 g four times daily, with steady-state mesalamine plasma concentrations

increasing about nine times, from 0.14 mcg/mL to 1.21 mcg/mL, suggesting saturable first-pass
metabolism. N-acetylmesalamine pharmacokinetics were linear.
Elimination. About 130 mg free mesalamine was recovered in the feces following a single 1-g PENTASA dose, which was comparable to the 140 mg of mesalamine recovered from the molar equivalent sulfasalazine tablet dose of 2.5 g. Elimination of free mesalamine and salicylates in feces increased proportionately with PENTASA dose. N-acetylmesalamine was the primary compound excreted in the urine (19% to 30%) following PENTASA dosing.
CLINICAL TRIALS
In two randomized, double-blind, placebo-controlled, dose-response trials (UC-1 and UC-2) of 625 patients with active mild to moderate ulcerative colitis, PENTASA, at an oral dose of 4 g/day given 1 g four times daily, produced consistent improvement in prospectively identified primary efficacy parameters.
The 4-g dose of PENTASA also gave consistent improvement in secondary efficacy parameters, namely the frequency of trips to the toilet, stool consistency, rectal bleeding, abdominal/rectal pain, and urgency. The 4-g dose of PENTASA induced remission as assessed by endoscopic and symptomatic endpoints.
In some patients, the 2-g dose of PENTASA was observed to improve efficacy parameters measured. However, the 2-g dose gave inconsistent results in primary efficacy parameters across the two adequate and well-controlled trials.
INDICATIONS AND USAGE
PENTASA is indicated for the induction of remission and for the treatment of patients with mildly to moderately active ulcerative colitis.
CONTRAINDICATIONS
PENTASA is contraindicated in patients who have demonstrated hypersensitivity to mesalamine, any other components of this medication, or salicylates.
PRECAUTIONS
General
Caution should be exercised if PENTASA is administered to patients with impaired hepatic function.
Mesalamine has been associated with an acute intolerance syndrome that may be difficult to distinguish from a flare of inflammatory bowel disease. Although the exact frequency of occurrence cannot be ascertained, it has occurred in 3% of patients in controlled clinical trials of mesalamine or sulfasalazine. Symptoms include cramping, acute abdominal pain and bloody diarrhea, sometimes fever, headache, and rash. If acute intolerance syndrome is suspected, prompt withdrawal is required. If a rechallenge is performed later in order to validate the hypersensitivity, it should be carried out under close medical supervision at reduced dose and only if clearly needed.
Renal
Caution should be exercised if PENTASA is administered to patients with impaired renal function. Single reports of nephrotic syndrome and interstitial nephritis associated with mesalamine therapy have been described in the foreign literature. There have been rare reports of interstitial nephritis in patients receiving PENTASA. In animal studies, a 13-week oral toxicity study in mice and 13-week and 52-week oral toxicity studies in rats and cynomolgus monkeys have shown the kidney to be the major target organ of mesalamine toxicity. Oral daily doses of 2400 mg/kg in mice and 1150 mg/kg in rats produced renal

lesions including granular and hyaline casts, tubular degeneration, tubular dilation, renal infarct, papillary necrosis, tubular necrosis, and interstitial nephritis. In cynomolgus monkeys, oral daily doses of 250 mg/kg or higher produced nephrosis, papillary edema, and interstitial fibrosis. Patients with preexisting renal disease, increased BUN or serum creatinine, or proteinuria should be carefully monitored, especially during the initial phase of treatment. Mesalamine-induced nephrotoxicity should be suspected in patients developing renal dysfunction during treatment.

Drug Interactions

There are no data on interactions between PENTASA and other drugs.

Carcinogenesis, Mutagenesis, Impairment of Fertility

In a 104-week dietary carcinogenicity study of mesalamine, CD-1 mice were treated with doses up to 2500 mg/kg/day and it was not tumorigenic. For a 50 kg person of average height (1.46 m2 body surface area), this represents 2.5 times the recommended human dose on a body surface area basis (2960 mg/m2/day). In a 104-week dietary carcinogenicity study in Wistar rats, mesalamine up to a dose of 800 mg/kg/day was not tumorigenic. This dose represents 1.5 times the recommended human dose on a body surface area basis.

No evidence of mutagenicity was observed in an in vitro Ames test and an in vivo mouse micronucleus test.

No effects on fertility or reproductive performance were observed in male or female rats at oral doses of mesalamine up to 400 mg/kg/day (0.8 times the recommended human dose based on body surface area).

Semen abnormalities and infertility in men, which have been reported in association with sulfasalazine, have not been seen with PENTASA capsules during controlled clinical trials.

Pregnancy

Category B. Reproduction studies have been performed in rats at doses up to 1000 mg/kg/day (5900 mg/M2) and rabbits at doses of 800 mg/kg/day (6856 mg/M2) and have revealed no evidence of teratogenic effects or harm to the fetus due to mesalamine. There are, however, no adequate and well-controlled studies in pregnant women. Because animal reproduction studies are not always predictive of human response, PENTASA should be used during pregnancy only if clearly needed.

Mesalamine is known to cross the placental barrier.

Nursing Mothers

Minute quantities of mesalamine were distributed to breast milk and amniotic fluid of pregnant women following sulfasalazine therapy. When treated with sulfasalazine at a dose equivalent to 1.25 g/day of mesalamine, 0.02 mcg/mL to 0.08 mcg/mL and trace amounts of mesalamine were measured in amniotic fluid and breast milk, respectively.

N-acetylmesalamine, in quantities of 0.07 mcg/mL to 0.77 mcg/mL and 1.13 mcg/mL to 3.44 mcg/mL, was identified in the same fluids, respectively.

Caution should be exercised when PENTASA is administered to a nursing woman.

No controlled studies with PENTASA during breast-feeding have been carried out.

Hypersensitivity reactions like diarrhea in the infant cannot be excluded.

Pediatric Use

Safety and efficacy of PENTASA in pediatric patients have not been established.

ADVERSE REACTIONS

In combined domestic and foreign clinical trials, more than 2100 patients with ulcerative colitis or Crohn's disease received PENTASA therapy. Generally, PENTASA therapy was well

tolerated. The most common events (ie, greater than or equal to 1%) were diarrhea (3.4%), headache (2.0%), nausea (1.8%), abdominal pain (1.7%), dyspepsia (1.6%), vomiting (1.5%), and rash (1.0%).

In two domestic placebo-controlled trials involving over 600 ulcerative colitis patients, adverse events were fewer in PENTASA-treated patients than in the placebo group (PENTASA 14% vs. placebo 18%) and were not dose-related. Events occurring at 1% or more are shown in the table below. Of these, only nausea and vomiting were more frequent in the PENTASA group. Withdrawal from therapy due to adverse events was more common on placebo than PENTASA (7% vs. 4%).

Nervous System: depression, dizziness, insomnia, somnolence, paresthesia

Cardiovascular: palpitations, pericarditis, vasodilation

Other: albuminuria, amenorrhea, amylase increase, arthralgia, asthenia, breast pain, conjunctivitis, ecchymosis, edema, fever, hematuria, hypomenorrhea, Kawasaki-like syndrome, leg cramps, lichen planus, lipase increase, malaise, menorrhagia, metrorrhagia, myalgia, pulmonary infiltrates, thrombocythemia, thrombocytopenia, urinary frequency

One week after completion of an 8-week ulcerative colitis study, a 72-year-old male, with no previous history of pulmonary problems, developed dyspnea. The patient was subsequently diagnosed with interstitial pulmonary fibrosis without eosinophilia by one physician and bronchiolitis obliterans with organizing pneumonitis by a second physician. A causal relationship between this event and mesalamine therapy has not been established.

Published case reports and/or spontaneous postmarketing surveillance have described infrequent instances of pericarditis, fatal myocarditis, chest pain and T-wave abnormalities, hypersensitivity pneumonitis, pancreatitis, nephrotic syndrome, interstitial nephritis, hepatitis, aplastic anemia, pancytopenia, leukopenia, agranulocytosis, or anemia while receiving mesalamine therapy. Anemia can be a part of the clinical presentation of inflammatory bowel disease. Allergic reactions, which could involve eosinophilia, can be seen in connection with PENTASA therapy.

Postmarketing Reports

The following events have been identified during post-approval use of the PENTASA brand of mesalamine in clinical practice. Because they are reported voluntarily from a population of unknown size, estimates of frequency cannot be made. These events have been chosen for inclusion due to a combination of seriousness, frequency of reporting, or potential causal connection to mesalamine:

Gastrointestinal: Reports of hepatotoxicity, including elevated liver enzymes (SGOT/AST, SGPT/ALT, GGT, LDH, alkaline phosphatase, bilirubin), hepatitis, jaundice, cholestatic jaundice, cirrhosis, and possible hepatocellular damage including liver necrosis and liver failure. Some of these cases were fatal. One case of Kawasaki-like syndrome which included hepatic function changes was also reported.

Other: Postmarketing reports of pneumonitis, granulocytopenia, systemic lupus erythematosis, acute renal failure, chronic renal failure and angioedema have been received in patients taking PENTASA.

OVERDOSAGE

Single oral doses of mesalamine up to 5 g/kg in pigs or a single intravenous dose of

mesalamine at 920 mg/kg in rats were not lethal.
There is no clinical experience with PENTASA overdosage. PENTASA is an aminosalicylate, and symptoms of salicylate toxicity may be possible, such as: tinnitus, vertigo, headache, confusion, drowsiness, sweating, hyperventilation, vomiting, and diarrhea. Severe intoxication with salicylates can lead to disruption of electrolyte balance and blood-pH, hyperthermia, and dehydration.
Treatment of Overdosage. Since PENTASA is an aminosalicylate, conventional therapy for salicylate toxicity may be beneficial in the event of acute overdosage. This includes prevention of further gastrointestinal tract absorption by emesis and, if necessary, by gastric lavage. Fluid and electrolyte imbalance should be corrected by the administration of appropriate intravenous therapy. Adequate renal function should be maintained.
DOSAGE AND ADMINISTRATION
The recommended dosage for the induction of remission and the symptomatic treatment of mildly to moderately active ulcerative colitis is 1g (4 PENTASA 250 mg capsules or 2 PENTASA 500 mg capsules) 4 times a day for a total daily dosage of 4g. Treatment duration in controlled trials was up to 8 weeks.
HOW SUPPLIED
PENTASA controlled-release 250 mg capsules are supplied in bottles of 240 capsules (NDC 54092-189-81). Each green and blue capsule contains 250 mg of mesalamine in controlled-release beads. PENTASA controlled-release capsules are identified with a pentagonal starburst logo and the number 2010 on the green portion and PENTASA 250 mg or S429 250 mg on the blue portion of the capsules.
PENTASA controlled-release 500 mg capsules are supplied in bottles of 120 capsules (NDC 54092-191-12). Each blue capsule contains 500 mg of mesalamine in controlled release beads. PENTASA controlled-release capsules are identified with a pentagonal starburst logo and PENTASA 500 mg or S429 500 mg on the capsules.
Store at 25°C (77°F) excursions permitted to 15-30°C (59-86°F) [see USP Controlled Room Temperature].
Manufactured for Shire US Inc. 725 Chesterbrook Blvd., Wayne, PA 19087, USA
Licensed U.S. Patent Nos. B1 4,496,553 and 4,980,173 189 0107 009
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Table 1. Adverse Events Occurring in More Than 1% of Either Placebo or PENTASA Patients in Domestic Placebo-controlled Ulcerative Colitis Trials. (PENTASA Comparison to Placebo)

Event	PENTASA n=451	Placebo n=173
Diarrhea	16 (3.5%)	13 (7.5%)
Headache	10 (2.2%)	6 (3.5%)
Nausea	14 (3.1%)	---
Abdominal Pain	5 (1.1%)	7 (4.0%)
Melena (Bloody Diarrhea)	4 (0.9%)	6 (3.5%)
Rash	6 (1.3%)	2 (1.2%)
Anorexia	5 (1.1%)	2 (1.2%)

Fever 4 (0.9%) 2 (1.2%)
Rectal Urgency 1 (0.2%) 4 (2.3%)
Nausea and Vomiting 5 (1.1%) ---
Worsening of Ulcerative Colitis 2 (0.4%) 2 (1.2%)
Acne 1 (0.2%) 2 (1.2%)