# Golimumab for Ulcerative Colitis
# Outcomes, Therapeutic Drug Monitoring, and Defining Conditions for Optimal Use

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*Awarding institution:*
King's College London

# Golimumab for Ulcerative Colitis:

# Outcomes, Therapeutic Drug Monitoring, and Defining Conditions for Optimal Use

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# Communications Arising from this Research

## Original Research Papers and Review Articles

- Golimumab: Early experience and medium-term outcomes from two UK tertiary IBD centres.

  M.A. Samaan, P. Pavlidis, J. Digby-Bell, E.L. Johnston, A. Dhillon, R. Paramsothy, A.O. Akintimehin, L. Medcalf, G. Chung-Faye, P. Dubois, I. Koumoutsos, N. Powell, S. Anderson, J. Sanderson, B. Hayee, P.M. Irving.

  *Frontline Gastroenterology*, July 2018. PMID: 30047549

  - Accompanying editorial:

    Golimumab for ulcerative colitis: Adding perspective to the pursuit.

    J.K. Limdi.

- Golimumab in the treatment of ulcerative colitis

  G.Cunningham, M.A. Samaan, P.M. Irving.

  *Therapeutic Advances in Gastroenterology*, January 2019. PMID: 30728858

- Therapeutic thresholds for golimumab serum concentrations during induction and maintenance therapy in ulcerative colitis: Results from the GO-LEVEL study.

  M.A. Samaan, G. Cunningham, A.G. Tamilarasan, L. Beltran, S. Ray, J. Mawdsley, S.H. Anderson, J.D. Sanderson, Z. Arkir, P.M. Irving.

  *Alimentary Pharmacology & Therapeutics*, June 2020. PMID: 32506695

  - Accompanying editorial:

    Therapeutic drug monitoring for golimumab – ready for prime time?

    X. Roblin, B. Le Roy, S. Paul.

## Oral Presentations

- Higher serum golimumab concentrations are associated with combined clinical-biochemical remission: Results from the GO-LEVEL study.

  British Society of Gastroenterology (BSG), Glasgow, 2019.

## Poster Presentations

- Golimumab: Early experience and medium-term outcomes from two UK tertiary IBD centres.

  BSG, Manchester, 2017.
- Therapeutic thresholds for golimumab serum concentrations during induction and maintenance: Results from the GO-LEVEL study.

  European Crohn's & Colitis Organisation (ECCO), Vienna, 2020

  Digestive Diseases Week (DDW), online, 2020

## Book Chapters

- 'Biologic Therapy of ulcerative colitis: Golimumab' featured in *Crohn's disease and ulcerative colitis: From epidemiology and immunobiology to a rational diagnostic and therapeutic approach*, Second Edition.

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# Abstract

Golimumab was approved by the US Food and Drug Administration and the European Medicines Agency for the treatment of moderate-to-severe ulcerative colitis in 2013 and was the third anti-tumour necrosis factor therapy, after adalimumab, and infliximab, licensed for this indication. These approvals were granted based on evidence generated by a large-scale, randomised controlled trial programme (PURSUIT) that demonstrated significant benefit compared with placebo during both induction and maintenance treatment. However, despite the efficacy demonstrated in PURSUIT, several aspects regarding the use of golimumab remained to be studied. These included its effectiveness in 'real-world' clinical practice, the exposure-response relationship associated with its use and the role that therapeutic drug monitoring may have to play in terms of treatment optimisation. In addition, as part of ascertaining a clearer understanding of the pharmacokinetics (PK) and pharmacodynamics (PD) of any drug, it is crucial that techniques used to measure serum concentrations are appropriately validated and verified.

This thesis provides an original contribution to knowledge, firstly by evaluating clinical outcomes of golimumab-treated patients as part of a retrospective observational study. Secondly, a prospective, phase IV, therapeutic drug monitoring study (GO-LEVEL) was designed to identify therapeutic thresholds for golimumab serum concentrations during induction and maintenance therapy. Finally, samples collected as part of GO-LEVEL were analysed to validate and verify use of a commercially available assay for measurement of serum golimumab and anti-golimumab antibody concentrations.

# Abbreviations

| | |
|---|---|
| 5-ASA | 5-aminosalicyclic acid |
| ADAb | Antidrug antibody |
| AGA | Anti-golimumab antibodies |
| ASUC | Acute severe ulcerative colitis |
| AUC | Area under the curve |
| AUROC | Area under the ROC curve |
| BMI | Body mass index |
| BSG | British Society of Gastroenterology |
| CD | Crohn's disease |
| CLIA | Chemiluminescent immunoassay |
| CRP | C-reactive protein |
| CV | Coefficient of variation |
| DBS | Dried blood spot |
| DDW | Digestive Diseases Week |
| ECCO | European Crohn's & Colitis Organisation |
| ECLIA | Electrochemiluminescence immunoassays |
| ELISA | Enzyme-linked Immunosorbent assay |
| EMA | European Medicines Agency |

| | |
|---|---|
| Fab | Fragment antigen binding |
| Fc | Fragment crystallisable |
| FC | Faecal calprotectin |
| FDA | Food and Drug Administration |
| FMT | Faecal microbiota transplantation |
| GETAID | Groupe d'Etude Thérapeutique des Affections Inflammatoires Digestives |
| GI | Gastrointestinal |
| GOL | Golimumab |
| GOUDA | A study investigating golimumab dried blood spot analysis |
| GSTT | Guy's & St Thomas' Trust Hospital |
| GTP | Guanosine-5'-triphosphate |
| HBI | Harvey-Bradshaw Index |
| HMSA | Homogenous mobility shift assay |
| HRA | Health Research Authority |
| IBD | Inflammatory bowel disease |
| IBDQ | Inflammatory Bowel Disease Questionnaire |
| IBDU | Inflammatory bowel disease unclassified |
| IgG | Immunoglobulin G |
| IISR | Investigator Initiated Sponsored Research |
| IMID | Immune-mediated inflammatory diseases |

| | |
|---|---|
| IOIBD | International Organisation for the study of IBD |
| JAK | Janus kinase |
| KCH | King's College Hospital |
| LTE | Long-term extension |
| MH | Mucosal healing |
| MSD | Meso Scale Discovery |
| MU | Measurement uncertainty |
| NHS | National Health Service |
| NICE | National Institute for Health and Care Excellence |
| OD | Optical density |
| PANTS | Personalised Anti-TNF Therapy in CD |
| PD | Pharmacodynamics |
| PIC | Patient identification centres |
| PK | Pharmacokinetics |
| PMS | Partial Mayo Score |
| PRO | Patient reported outcome |
| PURSUIT | Programme of Ulcerative Colitis Research Studies Utilising an Investigational Treatment |
| QoL | Quality of life |
| Rac1 | Ras-related C3 botulinum toxin substrate 1 |

| | |
|---|---|
| RCT | Randomised controlled trials |
| RES | Reticuloendothelial system |
| RIA | Radioimmunoassay |
| RLU | Relative light unit |
| RNA | Ribonucleic acid |
| ROC | Receiver operating characteristics |
| SAE | Serious adverse events |
| SAR | Serious adverse reaction |
| SCCAI | Simple Clinical Colitis Index |
| SES-CD | Simple endoscopic score for Crohn's disease |
| SFS | Steroid-free serum |
| SGC | Serum golimumab concentrations |
| SMART | An open-label observational study that explored patient preference for either pen or syringe to deliver golimumab in Belgium. |
| SST | Serum separator tubes |
| STRIDE | Selecting Therapeutic Targets in Inflammatory Bowel Disease |
| Th | T helper cells |
| TDL | Assay dilution buffer |
| TDM | Therapeutic drug monitoring |
| TL | Trough levels |

| | |
|---|---|
| TMB | 3,3’,5,5’-Tetramethylbenzidine |
| TNF | Tumour necrosis factor |
| TREAT | Crohn's disease Therapy, Resource, Evaluation, and Assessment Tool |
| UC | Ulcerative colitis |
| UCEIS | Ulcerative colitis endoscopic index of severity |
| VAS | Visual analogue scale |
| VBIC | Virtual biologics and immunosuppressives clinic |
| VICTORY | Vedolizumab health outcomes in inflammatory bowel diseases consortium |

# Chapter 1: Introduction

## Inflammatory Bowel Disease

### History, epidemiology, and pathogenesis.

IBD is an umbrella term which includes ulcerative colitis (UC), Crohn's disease (CD) and microscopic colitis. All three conditions are characterised by chronic inflammation of the gastrointestinal tract and although clear differentiations exist, there are also significant areas of overlap. The exact timing of the first report of IBD in medical literature is a matter of debate but there are descriptions of clinical syndromes consistent with the condition dating back to Greek antiquity (Mulder, Noble, Justinich, & Duffin, 2014). However, it was not until 1859 that Sir Samuel Wilks (a British physician, who studied and worked at Guy's Hospital) first described UC in terms that are similar to our modern-day understanding of the disease (Wilks, 1859). Although Sir Wilks' case report appeared to coin the term 'ulcerative colitis,' it was not until a more comprehensive report by Sir William Hale-White (also a physician at Guy's Hospital) in 1888 that the term entered the general medical vocabulary (White, 1909). The term 'Crohn's disease' was not coined until almost 50 years later, and in circumstances which remain unclear and controversial to this day, when the condition acquired the eponym of the first author of a report which included 14 patients with 'regional ileitis' (Crohn, Ginzburg, & Oppenheimer, 1932). Burrill B. Crohn co-authored the 1932 report with fellow American physicians, Leon Ginzburg, and Gordon D. Oppenheimer, apparently contributing only two patients to the cohort but being named first in the *Journal of the American Medical Association* author list by virtue of their alphabetical order policy at the time. Their description of the observed pathology was

characterised by chronic 'necrotizing and cicatrizing [healing by scar formation] inflammation' in the terminal ileum, as well as transmural inflammation, strictures, and fistulas. Although in hindsight previous case series (such as that by Thomas K. Dalziel in the *British Medical Journal* in 1913) were almost certainly earlier descriptions of CD (Dalziel, 1913), it was not until the seminal report by Crohn et al. (1932) that understanding of the disease truly began to crystallise.

The most common clinical manifestations of IBD include diarrhoea, rectal bleeding, abdominal pain, and weight loss. Extra-intestinal features are also common and can include systemic manifestations such as fever, malaise, and anorexia, as well as symptoms affecting the joints (arthritis/arthralgia), eyes (scleritis/episcleritis) and skin (erythema nodosum/pyoderma gangrenosum). The onset of symptoms can be insidious and initial misdiagnosis (often as irritable bowel syndrome) and/or late presentation to a clinician may result in diagnostic delay (Nguyen et al., 2017; Vavricka et al., 2012). In most cases, the disease follows a relapsing-remitting course, with periods of disease activity brought into remission of varying length by medical or surgical interventions (Solberg et al., 2009). However, predicting disease course and response to treatment is difficult (Liverani et al., 2016) and therefore, over the course of the last decade or so, there has been a concerted effort to try to understand these factors on an individual (rather than population) basis. These progressive efforts have begun ushering the field into the era of 'personalised' (Flamant & Roblin, 2018) and 'precision' (Denson et al., 2019) IBD medicine.

Most individuals affected by IBD develop symptoms and are diagnosed in young adulthood. Traditional epidemiological understanding then describes a second, smaller peak in the fifth to seventh decades (Calkins et al., 1984) resulting in a bimodal incidence pattern. In more

recent studies, however, this second peak has been absent (or much smaller than previously thought) and the incidence of UC has appeared to plateau throughout adulthood rather than peaking and falling away sharply (Johnston & Logan, 2008). The overall incidence of IBD was observed to be steadily increasing in the late 20th century (Loftus, 2004; Munkholm et al., 1992) and rates of prevalence now exceed 0.3% in North America and many European countries (Ng et al., 2018). However, more recent evidence suggests that rates may be stabilising (Ng et al., 2018) and it remains unclear how much of the observed increase was genuine and how much was owing to growing awareness, better reporting, and more sensitive diagnostic tools. Within Europe there exists a North-South gradient in incidence, but the gap appears to be narrowing with prevalence in Southern and Eastern European countries increasing at higher rates than those in the North (Shivananda et al.,1996). The prevalence of UC (238 per 100,000 adults) and CD (201 per 100,000 adults) appear comparable to one another (Kappelman et al., 2007), as does the incidence (UC: 2.2-14.3 cases per 100,000 person-years, CD: 3.1 to 14.6 cases per 100,000 person-years) (Loftus, 2004). The National Institute for Health and Care Excellence (NICE) estimates that IBD affects approximately 146,000 people in the UK and costs the National Health Service (NHS) in excess of £700 million per year (Bassi et al., 2004). The condition also has a significant impact upon quality of life (QoL) with 73% of UC patients reporting interference in their leisure activities, two-thirds describing a negative impact on their work, and over a quarter having to alter their work to accommodate their disease (Ghosh & Mitchell, 2007).

Even though these conditions have now been closely studied for a century or so using techniques of ever-increasing sophistication, their pathogenesis remains incompletely understood. Nonetheless, there have been significant advances and it is now generally

accepted that they occur in genetically susceptible individuals, exposed to environmental risk factors that result in dysregulation of the adaptive and innate immune responses and subsequent chronic gut inflammation (Bernstein et al., 2006; Lowe et al., 2009; Momozawa et al., 2018). Contemporary genetic sequencing techniques have allowed the identification of several loci that confer an increased risk of developing CD or UC (Momozawa et al., 2018; Verstockt, Smith, & Lee, 2018), whilst multiple causative environmental agents have been hypothesised and studied (Turpin et al., 2018). By far the most clinically significant of these was the well-established link between cigarette smoking and CD (Thomas et al., 2000). The fact that smoking conversely appears to have a beneficial effect in UC (Thomas et al., 2000) is an, as yet, unexplained oddity of IBD medicine and serves as a constant reminder that however similar, these conditions remain distinct from one another. Another example of this is the relationship observed between appendicectomy and IBD. An inverse association between appendicectomy and the subsequent development of UC has been consistently reported (Sahami et al., 2016). This observation has even led to the investigation of appendicectomy in the treatment of refractory UC (Sahami et al., 2019). Whilst there exists greater heterogeneity in the data regarding CD, there is some evidence suggesting that appendicectomy increases incidence, at least in the first post-operative year (Kaplan et al., 2008).

Understanding the link between gut microbiota and the development and/or propagation of gut inflammation currently appears tantalisingly close whilst overwhelmingly complex. For example, IBD associated dysbiosis has consistently been shown to include a reduction in biodiversity and an expansion of facultative anaerobic bacteria of the family Enterobacteriaceae (Zuo & Ng, 2018). However, despite novel techniques to carry out

detailed microbial evaluation, interventions aimed at its manipulation, such as antibiotics, probiotics, or more recently, faecal microbiota transplantation (FMT), remain relatively crude, and empirical. Playing into the notion of a microbial precipitant or propagator of IBD is the argument that accompanies the 'hygiene hypothesis.' This involves an attempt to explain why the improvement in hygienic conditions may lead to intestinal dysbiosis as a primary event, resulting in IBD amongst genetically predisposed individuals. The hypothesis implies that a lack of exposure to common infectious agents, believed to be necessary in programming the immune response of the gut, establishes an immunological imbalance between proinflammatory type 1 T helper (Th1) cells and tolerance-inducing regulatory T cells in early childhood (Amre et al., 2006). Subsequent exposure to pathogens is then postulated to be the event that triggers an inappropriate immunological response characterised by the development of an aberrant inflammatory process and potentially IBD.

Other environmental factors, including stress (Mawdsley & Rampton, 2005) and diet (Knight et al., 2015), are the focus of great interest (especially amongst patients) and offer the opportunity to both better understand and manage IBD. With regards to the former, observational studies have demonstrated that adverse life events, depression and chronic stress are associated with increased rates of relapse (Mawdsley & Rampton, 2005). Structured programmes have, therefore, been designed and trialled to directly address these issues, although their impact upon inflammatory activity remains to be seen (Wynne et al., 2019). With regards to diet, whilst no specific dietary precipitant has ever been identified, the increasing incidence of IBD in Asia has been used as a paradigm to observe environmental effects on the pathogenesis of immune-mediated disease. Change in dietary patterns, to mirror those more commonly seen in the West, has often been cited as the

main factor driving this in epidemiological observation (Yang, Owyang, & Wu, 2016). Despite the absence of an identifiable causative factor, the benefit of dietary manipulation in certain circumstances (e.g., active luminal CD) has been understood for many years (Narula et al., 2018a). However, there remains a paucity of high-quality evidence from which to recommend any specific diet for the maintenance of remission (Knight et al., 2015).

**Definition, classification, and natural history.**

The most recent and significant attempt to define and classify IBD was made by the Montreal Working Party at the 2005 World Congress of Gastroenterology. This multidisciplinary group reached consensus, which built upon previous similar exercises carried out in Rome (1991) and Vienna (1998) (Gasche et al., 2000). The Montreal classification, like its predecessor, considered age of onset (A), disease location (L), and disease behaviour (B) as the predominant phenotypic elements of CD. Age is categorised as A1 for those with age of diagnosis at 16 years or younger, whereas A2 and A3 account for age of diagnosis at 17–40 years and >40 years, respectively. The transmural inflammation seen in CD can affect any part of the gastrointestinal (GI) tract, from mouth to anus, and the Montreal classification divides location into, L1: ileal, L2: colonic, and L3: ileocolonic. L4 is an additional modifier and can be used alone or alongside the other locations when upper GI disease is present. Although no single unifying definition exists for CD, discontinuous segments of disease (‘skip lesions’) and granulomatous inflammation are suggestive, as is a tendency for inflammation to be worse in the proximal colon (Feakins, 2013). Disease behaviour is categorised as B1: non-stricturing, non-penetrating, B2: stricturing, and B3: penetrating, with p being used as a modifier to denote the additional presence of perianal disease (Satsangi et al., 2006; Silverberg et al., 2005). At diagnosis the majority have B1

disease; however, up to 20% of patients demonstrate a more aggressive phenotype, a figure which progresses to 51% at 20 years. This pattern of progression is particularly prevalent in those with ileal involvement and/or perianal disease (Langholz, 2010). Historically, approximately half of CD patients would require surgery at some point, with rates of disease recurrence similar in the subsequent decade (Peyrin-Biroulet et al., 2010), and approximately a third requiring further surgery (Frolkis et al., 2014). However, more recent evidence has suggested that the era of highly effective biologic agents has resulted in a positive effect in terms of reducing rates of surgery (Ma et al., 2017). Certain factors, identified by Beaugerie et al. (2006) were observed to predict a more severe disease course. These include a younger age at diagnosis, perianal disease, a need for corticosteroids at presentation, colonic resection, repeated small bowel resection, a stricturing phenotype, and substantial weight loss (Beaugerie et al., 2006).

The inflammation seen in UC is confined to the mucosa and involves a variable length of the colon, extending in a continuous manner from the rectum. No histological feature is diagnostic of UC, but the combination of basal plasmacytosis, diffuse crypt atrophy, and distortion, villous surface irregularity, and mucus depletion are suggestive of a diagnosis of UC in the correct clinical context (Feakins, 2013). The Montreal classification describes three different disease distributions, based on the maximal extent (E) of macroscopic disease at colonoscopy. E1 (‘ulcerative proctitis’) denotes involvement limited to the rectum, E2 (‘left-sided’ or ‘distal UC’) describes involvement limited to a proportion of the colorectum distal to the splenic flexure, and E3 (‘extensive UC’ or ‘pancolitis’) means involvement extends proximal to the splenic flexure (Satsangi et al., 2006; Silverberg et al., 2005). Figures regarding proportions of patients in each group vary, but E1, E2, and E3 rates were recently reported

from a large Italian cohort (n=1723) of 19.7%, 54.2%, and 26.1%, respectively (Manetti et al., 2016). The strengths of classifying UC patients based upon their disease extent include direct clinical relevance with regards to the use of topical therapies as well as prognostication. Disease extent is a predictor of disease severity, need for colectomy and the development of colorectal cancer (Hoie et al., 2007; Loftus, 2006). The life-time risk of colectomy is approximately 20-30%, increasing to 40% in patients with extensive and long-standing disease (Langholz et al., 1996). Conflicting reports exist regarding whether the advent of biologic treatments have had a meaningful impact on these figures (Manetti et al., 2016; Parragi et al., 2018). Many other predictors of an adverse UC disease course have been postulated. These include a period of less than two years from diagnosis to the first flare, the presence of fever or weight loss at onset, and active disease in the preceding 12 months (Langholz et al., 1996). The main weakness of an extent-based classification system is the appreciation that disease extent can change over time. The rate of proximal extension of proctitis over 10 years is estimated to be as great as 41-54% (Silverberg et al., 2005). Progression of left-sided colitis may be even higher. The contrary observation is also valid – that disease extent may regress over time (Safroneeva et al., 2015).

In approximately 5-15% of cases it is not possible to clearly define which IBD phenotype, UC, or CD, a patient best fits (Prenzel & Uhlig, 2009; Tremaine, 2007). In this case the term 'IBD unclassified' (IBDU) is used. As part of the Montreal classification, IBDU replaced the previous term 'indeterminate colitis,' which is now reserved for cases which cannot be confidently phenotyped even after undergoing colectomy and complete histopathological analysis (Silverberg et al., 2005). Approximately 75% of IBDU patients will maintain their unclassifiable status, whilst the remainder will be subsequently reclassified after developing

features more suggestive of either UC or CD (Birimberg-Schwartz et al., 2017). Despite efforts to better define it (Birimberg-Schwartz et al., 2017), IBDU remains a relatively poorly understood and understudied entity (Tremaine, 2007), with patients often being excluded from randomised controlled trials (RCTs).

**Conventional medical therapies.**

The cornerstone of UC treatment is mesalazine (also known as mesalamine and 5-aminosalicylic acid, 5-ASA), administered orally, and/or per rectum. It has proven efficacy for the induction and maintenance of remission in mild-to-moderate disease. Regardless of the route of administration, mesalazine acts topically in the colon and mucosal concentrations have been observed to correlate with efficacy (Frieri et al., 2000). Recent evidence suggests that its effect is mainly mediated by peroxisome proliferator-activated receptor (PPAR)-gamma; binding of mesalazine to this transcription factor causes its translocation to the nucleus and conformational change, which affects gene transcription. Other putative anti-inflammatory actions of mesalazine include modulation of inflammatory cytokine (TNF-alpha and IL-1) production, decreased transcriptional activity of nuclear factor-kappa beta by modulating RelA/p65 phosphorylation, and inhibition of the biosynthesis of prostaglandins and leukotrienes (Derijks et al., 2018).

Corticosteroid treatment in UC is generally reserved for acute severe flares, or those uncontrolled by high dose 5-ASA therapy alone. Depending on severity and disease extent, this can be administered intravenously (in the form of hydrocortisone or methylprednisolone), orally, or rectally as prednisolone or budesonide. Corticosteroid efficacy in UC was proven as long ago as 1955 (Truelove & Witts 1955). Corticosteroids diffuse into target cells and bind to a cytoplasmic glucocorticoid receptor. The receptor-

steroid complex undergoes a transformation that results in its activation, after which the receptor-steroid complex becomes capable of trafficking into a cell nucleus. Once within the nucleus, the complex is bound to a specific glucocorticoid regulatory element on target DNA molecules, initiating, or inhibiting gene transcription. This results in the synthesis of specific messenger RNA (mRNA) and consequently protein synthesis, which is eventually responsible for the glucocorticoid response (Thiesen & Thomson, 1996). This response, with respect to the anti-inflammatory and immunosuppressive actions of corticosteroids, typically includes decreased number, and activity of leukocytes in areas of acute inflammation, decreased activity of mononuclear cells, decreased proliferation of blood vessels and less fibrosis in areas of chronic inflammation, decreased clonal expansion of T and B cells and decreased action of cytokine-secreting T cells in lymphoid areas, decreased production and action of many proinflammatory cytokines, and reduced vasodilatation (Derijks et al., 2018). It is widely accepted that exposure to corticosteroids should be limited, given their lack of efficacy for maintenance of remission and high side effect profile. Therefore, patients requiring frequent corticosteroid therapy (more than two courses in a 12-month period), or in those with steroid dependent disease, escalation to a thiopurine is recommended (Mowat et al., 2011). However, in patients with disease that is refractory to steroids, escalation to a biologic agent is advocated because their onset of action is more rapid than that of the thiopurine agents (Harbord et al., 2017).

The thiopurine group of drugs includes azathioprine, mercaptopurine and tioguanine (which is less widely used than the former agents). They have proven efficacy in IBD and have been used and studied for many decades (Adler & Korelitz, 1990; Pearson et al., 1995). The process through which they suppress inflammation involves a complex set of genetic

activities, which involves the targeting of Rac1 (Ras-related C3 botulinum toxin substrate 1) activation. Through metabolism, azathioprine is converted into 6-mercaptopurine, which is then converted into 6-thio-guanine. 6-thioguanine is converted into two metabolites: one that is incorporated into DNA (6-thioguanine nucleotides), and one that is incorporated into small GTPases (6-thio-GTP).

Small GTPases play a role in various cell processes such as growth, differentiation, and movement. Rac1 is a member of the small GTPase protein family. One of the azathioprine metabolites, 6-thioguanine triphosphate (6-thio-GTP) binds to Rac1 as a competitive antagonist of guanosine-5'-triphosphate (GTP). This binding suppresses the activation of Rac1, which leads to apoptosis. Through its effect on Rac1 activity, therefore, azathioprine converts a co-stimulatory signal into an apoptotic signal (Tiede et al., 2003).

In clinical practice, the role played by thiopurines is limited by their delayed onset of action, a relatively narrow therapeutic index, inefficacy in one-third to one-half of patients, and intolerance (which requires treatment withdrawal in up to a third) (Jharap et al., 2010; Kennedy et al., 2013). However, through a great deal of research, the pharmacogenetics of thiopurine metabolism has become increasingly well understood and testing is now considered part of routine clinical care (Sanderson, 2015). This, in combination with the ability to monitor thiopurine metabolites and an appreciation of their importance has improved the safety, tolerability and effectiveness of this group of drugs (Haines et al., 2011).

Treatment strategies in CD are broadly similar to UC, with the exception of mesalazine, which appears ineffective for the induction or maintenance of remission (Akobeng et al., 2016; Lim et al., 2016), and inclusion of methotrexate. Despite being included in some UC

treatment algorithms (Harbord et al., 2017), the balance of evidence suggests that methotrexate provides no (or at best, very little) benefit for that indication (Carbonnel et al., 2016; Herfarth et al., 2018; Wang et al., 2015). It has, however, demonstrated evidence of benefit in CD (Feagan et al., 1995). Its effect is mediated through competitive antagonism of folic acid which, at high doses, produces a cytotoxic and antiproliferative effect by inhibiting dihydrofolate reductase and thus blocking DNA and RNA synthesis. The anti-inflammatory and immunomodulatory actions of low doses are probably due to inhibition of other folate dependent enzymes. Long-term low dose methotrexate may lead to accumulation of adenosine, a lymphotoxic, immunosuppressive, and anti-inflammatory autocoid. Other effects include interleukin 1 (IL-1) receptor blockade, increased production of the regulatory cytokine IL-2, decreased production of soluble IL-2 receptors, IL-6, IL-8, leukotriene B4, and antibodies, and impairment of neutrophil chemotaxis (Rampton, 2001).

It should also be noted that despite two recent, large, randomised studies that called into question the efficacy of thiopurines in CD (Cosnes et al., 2013; Panes et al., 2013), they currently still form an important part of treatment algorithms in most countries (Gomollon et al., 2017; Lamb et al., 2019).

A wide range of other immunosuppressive agents have been studied for the treatment of UC and/or CD with varying degrees of efficacy observed. These have included, but are by no means limited to, tacrolimus, ciclosporin, thalidomide, and mycophenolate. Although still used occasionally under specific circumstances, the era of biologic agents with favourable efficacy and safety profiles has rendered most of these agents redundant in the treatment of IBD.

**Novel small molecule therapies.**

Historically, small molecule treatments have formed the bedrock of IBD treatment. These low molecular weight (<900 daltons), organic compounds are synthesised by combining specific agents in a series of chemical reactions. Their use goes back as far as Nana Svartz's incidental finding in 1942 that sulphalazine not only treated rheumatoid arthritis but also improved symptoms in UC (Svartz, 1942). This was widely used in UC for many decades before it was superseded by mesalazine, which provides the same benefit with a favourable safety profile. Truelove and Witts' (1954, 1955) seminal trials of cortisone in the 1950s then extended the range of effective agents, whilst also demonstrating ability to improve mucosal appearances as well as symptoms. In fact, until the introduction of infliximab in the late 1990s, the treatment of IBD was entirely based on small molecules (as described above). However, since that time, virtually all novel treatments have been monoclonal antibodies produced by living systems, such as cell lines, and therefore, termed biologics. The advent of the use of these large, complex biologically derived molecules has undoubtedly moved IBD care into a new era. Biologics have been demonstrated to be highly effective agents that are able to deliver not only symptom resolution and improved QoL but also the ability to heal intestinal mucosa and perianal fistulation. In recent years, the range of available biologic mechanisms has expanded and in addition to TNF inhibition with infliximab, adalimumab, or golimumab, we now also have the ability to inhibit leukocyte trafficking to the gut with vedolizumab and proinflammatory interleukin-12 and -23 signalling with ustekinumab. Beyond these novel approaches, there is a range of monoclonal antibody therapies currently undergoing clinical trials and it appears clear that biologic agents will continue to play a key role in the treatment of IBD. However, there is

also now a renewed interest in small molecule therapies. Tofacitinib, a janus kinase (JAK) inhibitor, has recently been approved for use in UC and a range of other small molecules are soon to follow. These include other JAK inhibitors, which selectively inhibit specific JAK isoforms, and sphingosine-1-phosphate (S1P) modulators (e.g., ozanimod and fingolimod), which effectively trap lymphocytes in lymph nodes to reduce the number of circulating effector T cells. (Silva, Ortigosa, & Benard, 2010).

**Treatment targets and strategies.**

IBD treatments have traditionally focused on ameliorating symptoms (inducing remission), preventing disease flares (maintaining remission) and restoring QoL. Whilst these goals have not changed, in recent decades there has been a new emphasis on the additional direct demonstration of gaining control over inflammatory activity. This was largely driven by studies demonstrating that simply treating symptoms may not be sufficient to alter the natural history of these progressive diseases (Greenberg et al., 1996; Sandborn et al., 2005). These observations led to the notion that resolving inflammation at a mucosal level may be necessary to provide improvement in longer-term outcomes. The term ‘mucosal healing’ is now widely used to describe this novel treatment target and is predominantly driven by endoscopic assessments. However, the most optimal and valid way to define this remains a contentious issue with several different definitions in current use for both UC and CD (Neurath & Travis, 2012). Nonetheless, achieving mucosal healing (however defined) has been shown to predict favourable outcomes in both diseases (Baert et al., 2010; Froslie et al., 2007; Rutgeerts et al., 2006; Schnitzler et al., 2009). On this basis and in an attempt to define, for the first time, universally applicable criteria that could be used to judge the adequacy of treatment response, the International Organisation for the study of IBD (IOIBD)

ran an initiative called STRIDE (Selecting Therapeutic Targets in Inflammatory Bowel Disease). The outputs of this consensus exercise were that the goal of therapy should be the combination of clinical (or patient reported) as well as endoscopic remission (Peyrin-Biroulet et al., 2015). Table 1 presents a comparison of UC and CD clinical endoscopic remission.

**Table 1**
*STRIDE proposed, composite treatment targets for UC and CD*

| **Ulcerative colitis** | **Crohn's disease** |
|---|---|
| **Composite Target: Clinical remission AND Endoscopic remission** | |
| Clinical remission<br>• **Definition**: Resolution of rectal bleeding and diarrhoea/altered bowel habit<br>• **Assessment**: At a minimum of three months during active disease | Clinical remission<br>• **Definition**: Resolution of abdominal pain and diarrhoea/altered bowel habit<br>• **Assessment**: At a minimum of three months during active disease |
| AND | AND |
| Endoscopic remission<br>• **Definition**: Resolution of friability and ulceration at flexible sigmoidoscopy or colonoscopy (Mayo endoscopic score 0 or 1)<br>• **Assessment**: At three-month intervals during the active phase | Endoscopic remission<br>• **Definition**: Resolution of ulceration at ileocolonoscopy (or resolution of findings of inflammation on cross-sectional imaging in patients who cannot be adequately assessed with ileocolonoscopy)<br>• **Assessment**: At six- to nine-month intervals during the active phase |

The field of treatment targets in IBD is fast moving and endoscopic mucosal healing alone is unlikely to remain the ultimate target for long. Already, more novel and stringent targets are currently being proposed to achieve even tighter disease control. For example,

histological healing is emerging as a target for UC (Neurath & Travis, 2012) and transmural healing for CD (Panes & Rimola, 2018), each with their own purported benefits.

Going hand-in-hand with the definition of specific treatment targets is the movement towards the use of 'treat-to-target' algorithms in IBD. This strategy implies identification of a predefined target, followed by optimisation of therapy and regular monitoring until the target is achieved (Agrawal & Colombel, 2019). This has already been shown to confer a benefit in CD with the CALM trial demonstrating that timely escalation of therapy, on the basis of clinical symptoms combined with biomarkers (in this case faecal calprotectin: FC), results in better clinical and endoscopic outcomes than symptom-driven decisions alone (Colombel et al., 2018). Similar strategies have been studied for the use of endoscopic targets in UC and appear feasible and similarly beneficial (Bouguen et al., 2014). There is, however, evidence that uptake in some centres is limited (Bryant et al., 2018). Uptake would no doubt be improved by the substitution of FC for repeated endoscopies and this has been reflected as part of a recent update to the STRIDE recommendations (STRIDE II (Turner et al., 2021).

A predecessor to treat-to-target approaches was the investigation of CD treatment paradigms that instead of progressively increasing the depth of immunosuppression, ('step-up'), used early combination immunosuppressive ('top-down') regimens with thiopurines and anti-TNF agents (D'Haens et al., 2008). Although this resulted in certain identifiable benefits (Tsui & Huynh, 2018), most expert consensus recommendations currently favour an 'accelerated step-up' approach (Gomollon et al., 2017; Lamb et al., 2019).

## Biologic Agents

There is no doubt that the advent of biologic therapy with monoclonal antibodies has provided significant benefit for patients with UC and CD. Their use has resulted in improvement in clinical symptoms (Feagan et al., 2013; Feagan et al., 2016; Hanauer et al., 2002; Hanauer et al., 2006; Reinisch et al., 2011; Rutgeerts et al., 2005; Sandborn et al., 2012; Sandborn et al., 2013; Sandborn et al., 2014a; Sandborn et al., 2014b; Sands et al., 2014), endoscopic evidence of inflammation (Cholapranee et al., 2017; Sands et al., 2018) and QoL (Feagan et al., 2007; Feagan et al., 2017; Louis et al., 2013; Sands et al., 2018). Moreover, the magnitude of the benefit they can deliver has increased with our collective understanding of how best to use them, for example, the realisation that to derive their maximum benefit these agents should ideally be used in a continuous manner with 'maintenance' dosing, rather than an as part of an 'on demand' episodic regimen, as was originally practiced (Rutgeerts et al., 2004). Evidence demonstrating their diminishing benefit when used later in the disease course has also driven earlier introduction than was previously the case, thereby maximising their effectiveness. Another good example is the potential benefit of using monoclonal antibodies (particularly the anti-TNF agents) in combination with a conventional immunomodulator, such as azathioprine (Colombel et al., 2010; Panaccione et al., 2014). More recently, technology has evolved that allows the measurement of serum drug concentrations (Vande Casteele, 2017; Vande Casteele et al., 2014). The combination of evidence demonstrating an exposure-response relationship for these agents (Adedokun et al., 2017; Adedokun et al., 2018; Papamichael et al., 2017; Rosario et al., 2017) along with ability to carry out therapeutic drug monitoring (TDM), has

therefore opened up entirely new avenues for treatment optimisation (Papamichael & Cheifetz, 2016; Steenholdt et al., 2016).

In recent years, the range of agents and mechanisms of action has expanded rapidly. In addition to the long-standing anti-TNF agents, infliximab and adalimumab, a third agent, golimumab (GOL) was added to the class, receiving its NICE approval for the treatment of UC in 2015. At the same time, NICE approved the selective leukocyte adhesion molecule inhibitor, vedolizumab, for use in both UC and CD. Most recently in 2017, ustekinumab, a monoclonal antibody targeting the p40 subunit of interleukin-12 and interleukin-23, was granted NICE approval for use in CD. In addition to these new agents, the number of licensed treatments has been further expanded by the growing range of infliximab and adalimumab biosimilar agents available.

### Established anti-tumour necrosis factor agents.

#### *Infliximab.*

The use of an anti-TNF monoclonal antibody in IBD was first reported in 1993 as a case report in *The Lancet* (Derkx et al., 1993). Subsequently in 1997, an RCT confirmed the efficacy of infliximab (then called cA2) in CD (Targan et al., 1997). Infliximab's efficacy for CD and UC was further proven in the ACCENT and ACT trials, respectively (Hanauer et al., 2002; Rutgeerts et al., 2005; Sands et al., 2004). In the years since these landmark trials, vast amounts of clinical trial and observational effectiveness data have affirmed infliximab's central role in the treatment of IBD.

Standard dosing for infliximab induction is a 5 mg/kg intravenous infusion at weeks 0, 2, and 6 and then every 8 weeks thereafter. However, there are several ways that this dosing regimen can be modified to optimise an individual's therapy. In patients with low infliximab

trough levels (TL) (and an absent, or low titre antidrug antibodies) during maintenance therapy, intensifying infliximab dosing can improve clinical outcomes and increase the number of patients achieving clinical response (Vande Casteele et al., 2015a). This may be achieved either by increasing each infusion to 10 mg/kg, or by shortening the dosing interval to either 4 or 6 weeks. Ideally, decisions regarding dose adjustment should be made with the benefit of TDM, inclusive of antidrug antibodies (ADAb) measurement. This should be considered in the commonly encountered clinical scenarios for which dose intensification has a weaker rationale, for example, active disease due to the development of high titre antibodies with sub-therapeutic TLs (immune-mediated pharmacokinetic failure) or adequate TLs without antibodies (mechanistic/pharmacodynamic failure), which may warrant a change in therapy rather than dose intensification (Vande Casteele et al., 2017).

At the opposite end of the spectrum, patients in deep remission on infliximab maintenance with supra-therapeutic TLs can de-escalate their dosing, as the relapse rates are low (Paul, Roblin, & Peyrin-Biroulet, 2015; Vande Casteele et al., 2015a). Again, this may be done by lengthening the inter-dose interval or reducing the concentration of the infusion (if previously receiving 10 mg/kg). The TAXIT study showed that dose reduction (targeting a TL of 3-7 ug/mL) results in a similar proportion of patients in remission, but with a 28% reduction in the associated drug costs (Vande Casteele et al., 2015a).

In acute severe UC (ASUC), there are conflicting reports about the efficacy of an accelerated induction dosing schedule. It is clear that the severe and extensive inflammation in ASUC leads to increased faecal loss of infliximab and a result, inadequate clinical effect (Brandse et al., 2015). To try and address this issue, studies have investigated whether increased drug delivery (either via shorter intervals or via increased concentrations at each infusion)

improves outcomes. Gibson et. al. (2015) performed a single-centre, retrospective study in 2014 comparing standard induction dosing with accelerated dosing (3 doses given within a median of 24 days, IQR 21-29) and showed a lower early colectomy rate in the accelerated dosing arm. Notably, the colectomy rates converge after the induction period and are similar when measured at 2 years. The steroid-free remission rates at 1 year were not different between the two groups either (Gibson et al., 2015). A recent multicentre, retrospective study and meta-analysis compared standard induction dosing to accelerated dosing, here defined as 5 mg/kg at shorter intervals or upfront 10 mg/kg dosing. This study failed to show any difference in the colectomy rates between the 2 groups at 3, 6, 12, or 24 months. Within the accelerated dosing group, patients with upfront dosing of 10 mg/kg had a lower colectomy rate (in-hospital and at the 1- and 2-year mark) when compared with patients given 5 mg/kg at shorter intervals (Nalagatla et al., 2018). Large-scale, prospective trials are required to determine the optimal induction strategy in acute severe colitis, but there is some signal that very high levels are required in this subset of patients to combat the severe, systemic inflammatory burden, and faecal infliximab loss.

The immunogenicity of infliximab, and its clinical implications, have been well-established for some time. A recent review article, analysing 114 studies, reported that infliximab immunogenicity rates ranged from 0-65.3%, with slightly higher rates reported for CD than UC. In addition, the proportions of patients achieving and maintaining a response was lower in those patients with detected ADAb. Other outcomes, including adverse event data (including rates of infusion reactions) and trough infliximab levels were superior in those who did not develop ADAb (Vermeire et al., 2018).

Robust RCT data exist demonstrating that combination therapy with azathioprine achieves higher remission rates in both UC and CD (Colombel et al., 2010; Panaccione et al., 2014). Recent observational and randomised studies, in abstract form, have shown combination therapy reduces the rates of immunogenicity. A large, prospective, observational UK-wide study from the PANTS (**P**ersonalised **A**nti-T**N**F **T**herapy in CD) investigator consortium showed immunogenicity rates for infliximab (Remicade) of 26% at week 54 and 42% at 3 years (similar results were seen for the infliximab biosimilar CT-P13). These rates were reduced with immunomodulator use (HR = 0.37, p<0.0001) (Kennedy et al., 2018). Similarly, a recent randomised study showed that in those who failed adalimumab due to ADAb development, use of combination therapy (with azathioprine) when starting infliximab, significantly lowered the risk of immunogenicity (Roblin et al., 2018). The high rates of infliximab immunogenicity observed in the literature and the associated poorer clinical outcomes advocate strongly for the use of combination therapy wherever possible.

*TDM.*

As is now widely appreciated, TLs of infliximab have been shown to correlate with clinical response, mucosal healing, and clinical remission. The TAXIT randomised, controlled study established that targeting infliximab TLs to 3-7 ug/mL, resulted in more efficient use of the drug (Vande Casteele et al., 2015a). An analysis of TL thresholds showed a progressive reduction in the proportion of patients not achieving remission at lower levels. The rates reduced from 25% at a level ≥1ug/mL, to 15% for those with a level ≥3ug/mL, 8% for levels ≥5 ug/mL, and 4% for levels ≥7 ug/mL. Notably, when these thresholds were analysed separately for CD and UC, the proportion of patients not in remission at each threshold was higher for UC than CD.

A subset of patients that warrants separate discussion includes patients who suffer from fistulating disease. It has been shown that higher infliximab TLs are associated with perianal fistula healing in both adults and children (El-Matary et al., 2018; Yarur et al., 2017). Yarur et al. (2017) performed a cross-sectional study showing that median infliximab levels amongst patients with fistula healing were significantly higher than those without fistula healing (15.8ug/mL vs. 4.4ug/mL). There was a linear association between infliximab TLs and fistula healing, when levels were stratified by quartiles. In addition, the absence of ADAb was shown to correlate with healing. The optimal levels for fistula healing were ≥10ug/mL and some patients even required levels of ≥20ug/mL (Yarur et al., 2017). The take-away point here is that higher TLs than would usually be considered necessary for treating luminal disease, may be required to achieve fistula healing.

The recently reported Norwegian Drug Monitoring Study (NOR-DRUM) investigated infliximab outcomes using proactive TDM, compared with standard management in the absence of TDM, during induction (NOR-DRUM A) and maintenance (NOR-DRUM B). The outcome of NOR-DRUM A, remission at week 30, showed no benefit of a proactive TDM approach over standard management (Syversen et al., 2021a). During NOR-DRUM B dosing was increased if IFX levels were <2.1 mg/mL, reduced if the level was >10.0 mg/mL and left unchanged in the range 3.0 to 8.0 mg/mL. Investigators were allowed to use their judgment about whether to optimize dosing when drug levels were in the ranges 2.1 to 2.9 mg/mL and 8.1 to 10.0 mg/mL. The primary outcome was loss of response over the course of 52 weeks in patients who had already been on IFX for at least 30 weeks. Standard care was associated with a higher risk of disease worsening than the TDM arm over the 52-week trial

(hazard ratio, 2.1; 95% CI, 1.5-2.9) and more frequent formation of ADAb at concentrations considered to be clinically significant (15.0% vs 9.2%)(Syversen et al., 2021b)

***Adalimumab.***

Adalimumab followed closely behind infliximab in its drug development and is a well-established treatment of IBD. The initial CLASSIC 1 and 2, and CHARM trials demonstrated its efficacy in the induction and maintenance of remission in CD (Colombel et al., 2007; Hanauer et al., 2006; Sandborn et al., 2007). Subsequently, the ULTRA trials proved its efficacy in moderate-to-severely active UC (Sandborn et al., 2012). The largest 'real-world' observational effectiveness cohort in CD patients (n= 1189) reported significant retention rates for adalimumab treatment with 62% of patients remaining on the drug after 4 years (Tanaka et al., 2018). In terms of comparable clinical response rates to the original CHARM trial, retrospective analysis of 438 CD patients on adalimumab demonstrated that 31.6% of patients with follow-up between 1-3 years were in steroid-free remission, compared with 23% at 2 years in CHARM (Kamm et al., 2011).

Although the observational data in UC are slightly less robust, a recently published retrospective cohort study of 107 patients treated with adalimumab reported clinical remission rates superior to that of the drug development trials (Tursi et al., 2018). In this study 76.2% maintained remission at 12 months versus 30.9% of patients in ULTRA II at 52 weeks. However, this large discrepancy is probably explained by the fact that this cohort had lower median Mayo scores at baseline compared to the patients in the original development trials.

Currently, the approved dosing schedule for adalimumab is to give 160 mg followed by 80 mg two weeks later and then a subsequent maintenance dose of 40 mg every two weeks. Unfortunately, there is still a proportion of patients who do not respond to – or lose response

to – adalimumab. In patients who do not respond, increasing the dose frequency to weekly has been shown to be effective in recapturing response in both CD (Ma et al., 2014) and UC (Van de Vondel, 2018). However, to date, the data regarding dose escalation of adalimumab have been retrospective. To assess this prospectively, the SERENE-UC and -CD trials randomised patients to receive higher induction and maintenance doses to establish whether primary and secondary loss of response can be avoided by achieving and maintaining higher serum drug concentrations from the outset. In the maintenance phase of SERENE-CD, patients were randomized to 2 arms, a clinically adjusted arm, in which dose escalation was driven by Crohn's Disease Activity Index and CRP, and a TDM arm, in which patients with an ADA level <5 mg/mL were dose escalated, while those with a level of 5 to 10 mg/ mL were only dose escalated if they had clinically active disease or a high CRP. A significantly higher clinical remission rate at week 12 (62.3% vs. 51.5%; *p*=0.008) was observed but at week 44 of maintenance, there were no differences in any of the clinical or endoscopic outcomes between the 2 arms. However, it must be noted that dose escalation occurred in 26 of 92 and in 36 of 92 in the clinically adjusted and TDM-driven arms respectively (D'Haens et al., 2022). Similarly, in SERENE-UC clinical response rates at week 8 (47.1% vs. 40.0%; *p*=0.008) were higher in the high-dose group but did not continue into the maintenance phase. During maintenance, an exploratory TDM arm was included in which the TDM algorithm allowed adjustment from 40 mg ADA every other week to 40 mg weekly, the 2 doses explored in the main arms of the maintenance study. In addition, for patients requiring dose escalation who were already on 40 mg weekly, a one-time booster dose of 160 mg occurred. Higher cut-off levels for dose escalation were chosen for SERENE-UC compared with SERENE-CD. Dose escalation occurred in all patients with levels <10 mg/mL and in no patients with levels >20 mg/mL. Those with intermediate levels underwent dose adjustment if they also had rectal

bleeding. Dose escalation occurred in nearly 85% of the TDM cohort and was largely driven by drug levels. Serum ADA levels, perhaps unsurprisingly, therefore trended toward those seen in the ADA weekly dosing arm, and no benefit over this was seen in the TDM arm (Panes et al., 2022).

*Therapeutic Drug Monitoring.*

Higher serum drug concentrations are associated with better outcomes, not only clinical remission but also endoscopic healing and deeper histological remission (Yarur et al., 2016a). However, unlike for infliximab, there is evidence to suggest that it may not need to be a TL that is taken. Ward et al. (2017) performed a prospective, observational study on 19 patients with Crohn's disease on maintenance adalimumab; serum levels were taken at multiple intervals during the usual 14-day cycle. From this, the authors conclude that although ideally TLs should be taken, if a level of ≥4.9 µg/mL is detected during the first 9 days of dose, it can reasonably predict an adequate TL (Ward et al., 2017).

There are varying results from real-world cohort studies in terms of the effect of concomitant immunomodulator therapy on response rates to adalimumab. Previously it was felt that the addition of immunomodulator offered no additional benefit in terms of prevention of ADAb (Matsumotoet al., 2016). However, recent data have demonstrated that immunomodulators significantly reduce immunogenicity of adalimumab (HR=0.34, $p<0.0001$) (Kennedy et al., 2018).

**Novel biologics.**

***Vedolizumab.***

Vedolizumab is a recombinant, humanised monoclonal antibody that binds to alpha-4 beta-7 integrin molecules expressed on colon-specific lymphocytes. This binding prevents the

migration of lymphocytes into the gastrointestinal parenchyma and the resultant intestinal inflammation (Ward, Sparrow, & Roblin, 2018). This gut-selective mechanism of action is a point of difference to the anti-TNF biologic agents.

The US VICTORY consortium (Vedolizumab health outcomes in inflammatory bowel diseases) has published the largest real-world observational effectiveness cohort to date. Amongst 212 CD patients, the reported rate of clinical remission (defined as the complete absence of Crohn's disease-related symptoms) at 12 months was 35%. In addition, this remission rate should be understood in the context of this cohort's high (90%) prior anti-TNF inhibitor exposure rate (Dulai et al., 2016). In their UC cohort (n=321), the authors reported corticosteroid-free clinical remission rates of 37% (with 73% having prior exposure to anti-TNF therapy) (Narula et al., 2018b). These relatively high rates of prior anti-TNF use, compared to the rates in the GEMINI trials (48% in GEMINI 1 and 62% in GEMINI 2), are a marker of the complexity of patients seen in current clinical practice and may explain the marginally lower clinical response rates in observational studies compared to RCTs. High prior anti-TNF exposure has been described in numerous 'real-world' cohorts, including the GETAID cohort (99% in CD and 98% in UC) (Amiot et al., 2016) and a local cohort of two major UK tertiary referral centres (76%) (Samaan et al., 2017). Interestingly, the one factor in the VICTORY cohort that reduced the likelihood of deep remission (in both UC and CD) was prior anti-TNF exposure. In CD, the other factors included active perianal disease, severe disease activity, and smoking history.

Vedolizumab follows the same induction and maintenance dosing schedule as infliximab; intravenous infusion at weeks 0, 2, 6, and then every 8 weeks thereafter. However, unlike infliximab, the vedolizumab infusions are not weight-based and are a standardised 300 mg

per infusion. Notably, CD patients may benefit from an extra dose at week 10, if they have not yet had an adequate response at week 6. This is supported by analyses of the week 52 clinical remission rates from GEMINI II and the week 10 clinical remission rates from GEMINI III (Sandborn et al., 2013; Sands et al., 2014).

There is scope to escalate maintenance therapy, to every 4 or 6 weeks, in those who experience secondary loss of response to vedolizumab. A recent meta-analysis and systematic review estimated that the rates of secondary loss of response to vedolizumab amongst UC patients was 39.8 per 100 patient years and 47.9 per 100 patient years for CD patients (Peyrin-Biroulet et al., 2018). The same study reported on the efficacy of dose intensification (300 mg vedolizumab given every 4 or 6 weeks) to recapture response to vedolizumab. The data, pooled from four studies, were jointly reported for UC and CD patients and showed that 56 of 111 (50%) secondary non-responders recaptured response with escalation of vedolizumab dose. A recent cohort study also showed that dose escalation in patients with secondary loss of response to vedolizumab is effective in recapturing response in UC patients with reductions in Simple Clinical Colitis Index (SCCAI) and C-reactive protein (CRP) (Sierra Morales et al., 2018). Unlike anti-TNF therapy, however, TDM of vedolizumab is still a developing field and not yet a well-established part of the clinician's armamentarium in guiding dose adjustment.

*Combination or monotherapy?*

The rates of immunogenicity amongst patients treated with vedolizumab are remarkably low (<5%) and appear to be a transient phenomenon (Feagan et al., 2013; Sandborn et al., 2013; Sands et al., 2014; Ward, Sparrow, & Roblin, 2018). The rate of ADAb development in GEMINI 1 was 3.7%, but only 1% had measurable antibodies on subsequent tests. For

GEMINI 2, the rate was 4.1%, and persistent antibodies 0.4%, and in GEMINI 3 the rate was 1% and persistent ADAb were found in no patients. A caveat to these low rates is that these tests were performed using a drug-sensitive assay (making it more difficult to detect antibodies in the presence of a drug). The low rates of immunogenicity have been corroborated in subsequent studies, even when testing samples with drug-tolerant assays (Ungar et al., 2018). Antibodies to vedolizumab during the induction phase (levels measured at week 2, 6 and 14) were identified in seven of 41 patients (17%), three of whom still responded to vedolizumab induction therapy and four of whom did not. Antibodies were detected in 2 of 60 patients (3%) in the maintenance phase of vedolizumab therapy. Taken together, this suggests that the presence of antibodies has minimal impact on clinical outcomes and is often transient. A further study using a drug-tolerant assay showed that four of 179 (2.2%) vedolizumab-treated patients developed antibodies after the first infusion but this had no correlation to subsequent drug TLs or need for dose optimisation. Of note, all were undetectable by week 40 (Bian et al., 2017).

Converse to what has been observed with anti-TNF agents, concomitant immunomodulation did not affect vedolizumab clearance or concentration in a study analysing population PK and PD (Rosario, 2017). This reinforces the notion that the use of concomitant immunomodulators solely to prevent immunogenicity is not necessary with vedolizumab. If concomitant immunomodulation is to be stopped, we recommend delaying its cessation until after the induction period, to prevent a deterioration of symptoms (given vedolizumab's slow onset of action). The use of calcineurin inhibitors (tacrolimus, cyclosporin) as a bridging agent during induction was shown to be a reasonable strategy in a small cohort of CD and UC patients commencing vedolizumab (Christensen et al., 2018).

*TDM.*

Data are emerging regarding the exposure-response relationship for vedolizumab, but the overall picture is still not entirely clear. Post-hoc analyses from all three GEMINI studies showed an exposure-response relationship for vedolizumab, for both UC, and CD. Quartile analyses of drug levels showed those in the highest quartiles had significantly higher rates of clinical response and remission during the induction and maintenance periods when compared to those in the lowest quartiles (Feagan et al., 2013; Sandborn et al., 2013; Sands et al., 2014). Studies have suggested that a week 6 TL is a potential predictor of the need for dose escalation (Williet et al., 2017; Yacoub et al., 2018). In a study examining the relationship between TLs at week 2, 6 and 14 and rates of mucosal healing at week 52, only week 6 TLs correlated with rates of mucosal healing. Week 6 drug levels of 18 ug/mL and above correlated with higher rates of mucosal healing (Yacoub et al., 2018). Another study showed that week 6 levels <19 ug/mL were associated with the subsequent need for dose escalation (Williet et al., 2017). These levels need further validation in larger studies and currently there is insufficient evidence to suggest a target therapeutic window for vedolizumab therapy.

A recently presented abstract of a randomised trial (ENTERPRET) examined the benefit of vedolizumab dose escalation in patients with high drug clearance during induction in UC. Drug levels were measured at week 5 and patients who had levels of 30-50ug/mL were given twice the standard dose (300mg every 4 weeks, regimen A). Those who had levels of <30ug/mL were given four times the standard dose (600mg every 4 weeks, regimen B). The primary endpoint was endoscopic mucosal healing at week 30. There was no difference in the different treatment arms (18.9% of patients in the standard-dose arm vs 14.5% of

patients in the combined dose-optimization arms (p=0.561). Rates of endoscopic mucosal healing at week 30 were also similar when standard dosing was compared with regimen A (p=0.612) and regimen B (p=0.666)(Yarur et al., 2022).

***Ustekinumab.***

Ustekinumab is the most recently NICE approved monoclonal antibody for the treatment of CD and UC. Approval was granted in 2017 for CD and 2020 for UC based on proven efficacy, both in anti-TNF naïve, and exposed patients, generated in the UNITI, and UNIFI trial programmes, respectively (Feagan et al., 2016; Sands et al., 2019b). Ustekinumab is a monoclonal antibody to the p40 subunit of interleukin-12 and interleukin-23 and offers another novel mechanism of action for IBD patients. In terms of observational effectiveness data for CD, there is a rapidly expanding body of evidence, much of which is in abstract form and uses variable timing and outcome definitions. Based on the results of a recent and comprehensive systematic review and meta-analysis, anywhere from approximately a quarter, up to nearly a half of patients can be expected to achieve a response in real-world clinical practice (Honap et al., 2021).

The dosing regimen for ustekinumab is unlike that of the other biologics in so far as it obliges an assessment of response to induction therapy in order to guide maintenance dosing. The induction doses are fixed: an intravenous administration equating to approximately 6 mg/kg at week 0 followed by a subcutaneous 90 mg dose at week 8. Thereafter, 90 mg subcutaneous maintenance dosing is given on an 8- or 12-weekly basis depending on response. It is recommended that patients are reviewed in the week/fortnight running up to week 16; for those achieving an ‘adequate response’ 12-weekly dosing should be commenced (next administration will therefore be at week 20), whilst those only

achieving a partial response should be given 8-weekly dosing (next administration at week 16). In patients with no response whatsoever (or deterioration), treatment withdrawal could be considered. However, as options in this scenario are often limited and late response is a recognised phenomenon (Sands et al., 2017), continuing 8-weekly dosing for another dosing cycle beyond week 16 may also be appropriate. Judging the adequacy of response may be aided by the use of paired Harvey-Bradshaw Index (HBI) scores and objective markers of inflammation (e.g., CRP and FC) at baseline and pre-week 16. If there is any uncertainty regarding dosing frequency, there is a good rationale to err on the side of over treatment, with 8-weekly dosing appearing to result in higher rates of endoscopic response and healing than 12-weekly dosing (Adedokun et al., 2018; Rutgeerts et al., 2016). At any point onward, the dosing interval can be altered, either from 12- to 8-weekly for loss of response – with both post-hoc RCT (Adedokun et al., 2018; Sands et al., 2017) and observational (Ma et al., 2017) evidence to support that manoeuvre – or from 8- to 12-weekly in sustained remission (although the effect of this has not been well characterised).

*Combination or monotherapy?*

A comprehensive pharmacokinetic and pharmacodynamic post-hoc analysis of the UNITI trials has been carried out, which offers valuable practical insights into several aspects of the best use of ustekinumab (Adedokun et al., 2018). First, even using a drug-tolerant assay (which is therefore able to detect ADAb in the presence of a drug) ustekinumab appears to result in very low rates of immunogenicity: just 2.3% among 1366 patients over a year of treatment. To put this into context, this would compare with rates of 11% for adalimumab and 26-28% for infliximab (Kennedy et al., 2018). Second, perhaps due to the observed low rate of immunogenicity and again in contrast to anti-TNF agents, the use of concomitant

immunomodulation did not significantly impact on ustekinumab drug levels (Adedokun et al., 2018). On this basis, where immunomodulators have failed, and are being used *only* to reduce immunogenicity, it may be more appropriate to discontinue their use and give ustekinumab as monotherapy.

*Therapeutic Drug Monitoring.*

The clearly observed exposure-response relationship for ustekinumab means that TDM is almost certainly going to become a useful clinical tool in the future, and therapeutic thresholds have already been postulated (Adedokun et al., 2018). Although CE marked assays are available, correlation between them and therapeutic thresholds are yet to be established in an IBD setting. Hence TDM for ustekinumab is not yet widely adopted in routine practice and dose adjustments are, therefore, currently carried out on an empiric basis.

**Biosimilar agents.**

Due to the way in which they are produced, that is by living cells, biologic drugs do not have generic but rather 'biosimilar' forms. It is important to note that these are large, complex molecules, and although they are highly similar, they are not identical to each other and so should not be used interchangeably. Previously, there was much caution regarding switching patients from the originator drug to its biosimilar form. However, the evidence to date suggests that doing this does not result in loss of response to therapy. Jorgensen et al. (2017) conducted a multicentre, randomised, and double-blinded trial in Norway that explored the effect of switching from originator to biosimilar across multiple different diseases including CD, UC, spondyloarthritis, rheumatoid arthritis, psoriatic arthritis, and psoriasis. The study comprised a total of 482 patients, including 155 patients with Crohn's

disease and 93 patients with UC. The primary outcome was disease worsening, which occurred in 26% of patients who continued on the originator and 30% of patients who switched to the biosimilar drug.

### Emerging biologics agents.

The rapid expansion of available biologic agents is set to continue with a range of novel agents already in the later stages of clinical trials, including a wave of agents that target the p19 subunit of interleukin-23. This mechanism can be thought of as a variation on that of ustekinumab, which inhibits signalling via interleukin-12 as well as -23. There are currently three agents proceeding through the various phases of clinical trial development; rizankizumab, guselkumab, and mirikizumab. All three agents have demonstrated benefit in other idiopathic inflammatory conditions such as psoriasis and psoriatic arthritis.

#### *Anti-TNF mechanism of action.*

Growing understanding of the molecular pathogenesis of immune-mediated inflammatory diseases (IMIDs) in the 1990s led to the appreciation of the pivotal role played by tumour necrosis factor alpha (TNFα) (Keffer et al., 1991). Its name originated from its initial description in 1975 as an endotoxin-induced glycoprotein, which caused haemorrhagic necrosis of solid tumours (Carswell et al., 1975). It was subsequently discovered to exist in both transmembrane (tmTNF) and soluble (sTNF) forms, both of which are biologically active (Silva et al., 2010). The increasing recognition of its importance led to the experimental use of monoclonal antibodies developed to prevent the interaction between this key inflammatory cytokine and its receptors (therefore, subsequently described as 'anti-TNF' agents, or sometimes TNF antagonists). TNFα has been demonstrated to induce cell proliferation and differentiation, and its signalling pathways regulate gene expression and

upregulate adhesion molecules (ICAM-1 and LFA-1) (Baert et al., 1999). It is a potent inducer of the inflammatory response, a key mediator of innate immunity, and plays an important role in the regulation of Th1 immune responses against intracellular bacteria and certain viral infections (Silva et al., 2010). However, dysregulation of these precariously balanced and complex systems is now understood to contribute to the pathogenesis which underlies a range of IMIDs.

Despite the unequivocal efficacy of anti-TNF agents in IBD and other IMIDs, there remains uncertainty regarding their precise mechanism of action. Putative mechanisms have been attributed to downstream events that result from the antagonism of interactions between sTNF and its receptor, as well as those mediated by inhibitor binding to tmTNF (Silva et al., 2010). These two mechanisms are not mutually exclusive and have been demonstrated to result in a wide range of anti-inflammatory effects. These include downmodulation of cytokine production and expression of adhesion molecules (reducing cell recruitment), apoptosis of monocytes and T cells, changes in the immune response regulation, antibody- or complement-dependent cell-mediated cytotoxicity, and reverse signalling through ligation with tmTNF (Silva et al., 2010).

### *Pharmacokinetics of anti-TNF agents.*

The PK of anti-TNF agents in IBD is complex and despite being extensively studied, still not understood in its entirety. There also exists substantial interindividual heterogeneity, the significance of which has only relatively recently been recognised. There are PK factors and mechanisms that are generalisable to any monoclonal antibody, given to any individual

(including healthy volunteers). There are, however, other mechanisms that specifically relate to the high burden of inflammation associated with IBD as well as the fact that the organ involved is, in effect, an interface with the outside world. Other key specific determinants that have emerged include the development of ADAb, serum albumin concentration, IBD phenotype, body mass index (BMI), gender and coadministration of immunosuppressive agents (Khanna et al., 2014).

Monoclonal antibodies generally exhibit two distinct catabolic pathways. The first is a nonspecific, linear (first-order) clearance pathway mediated by interaction between the fragment crystallisable (Fc) region of the antibody and Fc receptors. Fc-mediated elimination is a common pathway shared by both endogenous immunoglobulin G (IgG) and therapeutic IgG monoclonal antibodies, which involves proteolytic catabolism via the reticuloendothelial system (RES). It is thought that RES activity is upregulated by inflammatory activity (Khanna et al., 2014) and the way in which it 'chews' through therapeutic antibodies has been conceptually described as a 'shark' (Rosen, Minar, & Vinks, 2015). Internalisation and subsequent degradation by lysosomes occurs after binding of the Fc portion to Fc receptors expressed on the surface of macrophages, natural killer cells, B and T cells and platelets (Comber et al., 1989). These receptors exist in several forms: Fc-gamma-receptor (Fc$\gamma$R) I, II and III, as well as neonatal Fc receptors (FcRn, also known as Brambell receptors, after their discoverer Prof Francis William Rogers Brambell (Brambell, Hemmings & Morris, 1964; Junghans, 1997). As opposed to clearance, Brambell receptors have actually been observed to play an important role in monoclonal antibody salvage and recirculation as well as the maintenance of endogenous immunoglobulin and albumin homeostasis. This function has been shown to prevent catabolism and results in a

prolongation of their half-life (Telleman & Junghans, 2000). These receptors become saturated at high concentrations of IgG antibodies or albumin. Although the precise mechanism responsible for the relationship between low albumin concentrations and accelerated drug clearance is unknown, one possibility is the development of enhanced binding of Brambell receptors to albumin in response to hypoalbuminemia, resulting in greater protein catabolism of globulins, including monoclonal antibodies (Khanna et al., 2014). Similarly, high levels of endogenous IgG, as are commonly observed in IBD, could reduce the half-life of exogenously administered monoclonal antibody agents (Ordas et al., 2012).

The second clearance pathway is nonlinear (target-mediated) and mediated by the specific interaction between the fragment antigen binding (Fab) region of the antibody and its pharmacological target. In the case of anti-TNF agents, this is TNFα (Dirks & Meibohm, 2010). This clearance pathway is often referred to as the 'antigen sink' (Khanna et al., 2014; Mould, 2015), or alternatively the 'antigen sponge' (Rosen et al., 2015). It is associated with disease severity and may saturate standard doses of TNF antagonists, resulting in inadequate tissue drug concentrations and poor control of inflammation. This concept is supported by the inverse correlation observed between plasma sTNF concentrations and clinical efficacy of TNF antagonists in rheumatoid arthritis (Takeuchi et al., 2011). This phenomenon appears to be equally true for concentrations of TNF in the gut mucosa of patients with IBD. For example, pre-treatment mucosal TNF gene expression inversely correlated with the likelihood of achieving clinical or endoscopic remission in a group of patients with active UC treated with infliximab (Olsen et al., 2009). Mucosal healing was achieved in 82%, 64% and 42% of patients with low, middling, and high pre-treatment

mucosal TNF gene expression, respectively. Similarly, amongst a mixed cohort of UC and CD, mucosal TNF concentrations were observed to be significantly elevated in areas of active inflammation and moreover, the ratio between TNF and anti-TNF (infliximab or adalimumab) in those areas was also elevated. This suggests that local tissue inflammation characterised by high levels of TNF may serve as a second sink for anti-TNF agents (Yarur et al., 2016b). Furthermore, this study identified a group of patients who had adequate serum anti-TNF levels but inadequate tissue concentrations to neutralise locally produced TNF (Yarur et al., 2016b). This not only offers an explanation for anti-TNF non-response in the presence of seemingly adequate serum levels, but also suggests a potential role for mucosal TNF levels as a surrogate pharmacodynamic marker that can be used to tailor treatment to the individual patient (i.e., personalised/precision medicine).

BMI and gender have been postulated as determinants of anti-TNF PK. However, these relationships are difficult to fully elucidate in view of the fact that these two factors confound one another (Khanna et al., 2014); male gender is associated with both greater drug clearance and greater weight (Fasanmade et al., 2009). The association between increasing weight and drug clearance is not linear and weight-based dosing (as the case for infliximab), therefore does not reliably predict drug exposure (Fasanmade et al., 2009). The production of proinflammatory cytokines by adipose tissue has been proposed as a potential mechanism to explain the higher drug requirements and occurrence of treatment failure observed in obese patients (Peyrin-Biroulet et al., 2007).

Regardless of the degree of humanisation, all monoclonal antibodies are exogenous proteins (antigens) and as such, can lead to sensitisation of the humoral immune system. This protective system is designed to generate high affinity antibodies against specific

microbial proteins. Through antigen (drug) recognition and B-cell receptor activation, there begins a process of cell proliferation and antibody production, with the drug as its target (Rajewsky, 1996; Rolink et al., 2001). These immune responses (and their resulting antibodies) are classed as 'neutralising' or 'non-neutralising' depending on their effect on the activity of the drug (Mould & Green, 2010). They have the potential to affect the drug activity in a number of ways. Firstly, they can lead to the formation of immune complexes that, owing to their large size, result in accelerated drug clearance – as much as threefold higher (Ternant et al., 2008) – and suboptimal serum drug concentrations (Schellekens & Casadevall, 2004). Secondly, direct neutralisation of biologic activity occurs through binding with the biologically active portion of the antibody. Thirdly, immune-mediated adverse reactions are triggered, such as serum sickness, that necessitate discontinuation of therapy (Gamarra et al., 2006).

Multiple factors have been described that influence the rate of ADAb development. These include individual genetic determinants (Sazonovset al., 2018), treatment related factors (including dosing regimen, stage of treatment, and route of administration), the characteristics of the particular drug (such as formulation, stability and degree of humanisation), and the use of concomitant immunosuppressive agents. Regarding treatment related factors, antibody formation has been observed to occur more frequently in the early phase of treatment (within 6-12 months of initiation: Bartelds et al., 2011) compared with patients on long-term maintenance. Very early formation is also recognised, within the induction period (Brandse et al., 2016). The intravenous route is generally considered less immunogenic than subcutaneous (Mould & Green, 2010). Each individual drug has its own immunogenicity profile and although lower with humanised agents, it is

worth noting that all therapeutic monoclonal antibodies approved to date have shown some immunogenicity (Mould & Green, 2010). There is a wealth of literature on the effectiveness of co-administering conventional immunosuppressive agents (such as a thiopurine or methotrexate) with biologic drugs to prevent immunogenicity (Hindryckx et al., 2017). They have even been demonstrated to reverse the formation of ADAb after they have been detected (Ungar et al., 2017) (see Table 2).

**Table 2**
*Infliximab, adalimumab and golimumab treatment data*

| | **Infliximab** | **Adalimumab** | **Golimumab** |
|---|---|---|---|
| **Bioavailability (%)** | 100 | 64 | 53 |
| **Maximum concentration (ug/mL)** | 118 | 4.7 +/- 1.6 | 3.1 +/- 1.4 |
| **Time to reach maximum concentration** | <1 hour | 5.46 +/- 2.3 days | 2-6 days |
| **Volume of distribution (L, assuming 70 kg body weight)** | 4.5-6.0 | 4.7-6.0 | 4.1-8.8 |
| **Half-life (days)** | 7.8-12.4 | 10-20 | 12 +/- 3 |
| **Clearance (mL/h)** | 15.3-18.4 | 11-15 | 20.1 +/- 5.8 |

The aforementioned monoclonal antibody clearance determinants are all generalisable, to a lesser or greater extent, across any disease process. However, by virtue of the GI tract injury that characterises IBD, it has an additional route of monoclonal antibody elimination that is unique. There is now increasing recognition that therapeutic monoclonal antibodies may also pass through the diseased gut mucosa into the stool. This protein-losing

enterocolopathy partially explains the hypoalbuminemia commonly observed in severe disease. The presence of detectable levels of a drug in the stool was first described in 2013 amongst a cohort of patients with active IBD, receiving infliximab induction therapy (Brandse et al., 2013). In a follow-up study of patients with moderate-to-severely active UC, they reported that those without endoscopic response at week 6-8 exhibited higher day 1 faecal infliximab concentrations, lower serum infliximab levels at week 6, and in some cases, early development of antibodies to infliximab (Brandse et al., 2015). This finding has since been reported elsewhere, including with the use of adalimumab and for CD as well as UC (Roblin & Paul, 2015). The presence of detectable drug in stools was observed to associate with markedly elevated FC and the presence of colonic ulcerations; both of which are recognised markers of disease severity. The phenomenon of stool loss of therapeutic antibodies has been conceptually described as a ‘sieve’ (Rosen et al., 2015: see Figure 1).

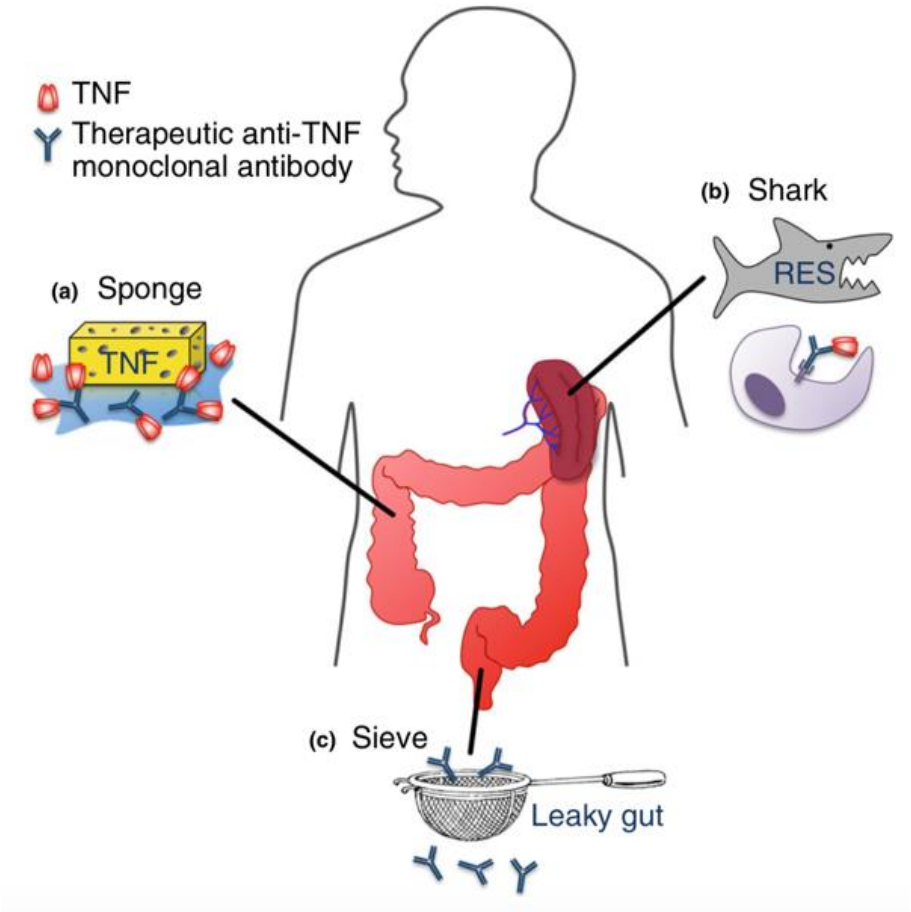



*Figure 1.* 'Sieve' concept of loss of drug through stools (TNF, tumour necrosis factor; RES, reticuloendothelial system)

***Challenges in the practical utility of TDM for infliximab and adalimumab.***

A myriad of challenges faces the clinician when trying to use current evidence of anti-TNF pharmacokinetics in the context of clinical practice. Moreover, these of course, have to be taken in conjunction with multiple patient and disease-related factors. We, therefore, hypothesised that there would be a great deal of heterogeneity in the way in which TDM for infliximab and adalimumab is a) utilised and b) interpreted. To test this hypothesis, we designed a survey that included five TDM-based clinical scenarios, for which the 'most appropriate' responses were based on the Building Research in IBD Globally (BRIDGe) group's 'Anti-TNF Optimizer' (http://www.bridgeibd.com/anti-tnf-optimizer: BRIDGe, 2011). This resource combines available TDM evidence with expert consensus. A link to our online

survey tool was sent to various IBD clinician groups in June 2017 including members of the British Society of Gastroenterology, Royal College of Nursing IBD Network, and the gastroenterology special interest group of the UK Clinical Pharmacy Association. We received 142 responses. Of these, 110 (77%) were complete, comprising 50 (45%) consultants, 30 (27%) trainees, 25 (23%) IBD nurse specialists and 5 (5%) gastroenterology pharmacists; these data were used for analysis.

Over half (61, 55%) only carry out TDM in non-response. The remainder use TDM routinely, including during stable maintenance therapy for patients in remission. Only 15 (14%) respondents reported being clear and confident in their understanding of the difference between drug-sensitive and drug-tolerant antibody assays. Moreover, most (82, 75%) were unsure as to which type their laboratory uses. Lower therapeutic thresholds used by clinicians were variable. Consultants, who were high-frequency TDM users (>3 requests/month) and clinicians with larger anti-TNF cohorts (>100 patients) were significantly more likely to select the 'most appropriate' answer to at least one of the five TDM scenarios (Samaan et al., 2018a).

#### *Challenges in measuring drug and ADAb concentrations.*

The longest-standing and most commonly employed technique for measuring drug and ADAb concentrations is by using an enzyme-linked Immunosorbent assay (ELISA). Alternative techniques include radioimmunoassays (RIA), homogenous mobility shift assays (HMSA), and chemiluminescence immunoassays (CLIA). In addition to the various techniques available, each may have multiple differing kits available (produced by different manufacturers), each with their own unique operating characteristics and until recently no international standardisation (Metcalfe et al., 2017).

Unfortunately, the situation is less straight-forward still with regards to the measurement of ADAb. For example, some assays (the first-generation bridging ELISAs) are only able to identify the ADAb in the absence of detectable drug in the serum (or at best, only with very low levels); these are therefore, described as 'drug-sensitive assays' and the antibodies they measure as 'free' antibodies (i.e., they are not part of drug- ADAb complexes). 'Drug-tolerant' assays (modified ELISAs, RIAs and HMSAs) can detect ADAb in the presence of therapeutic drug levels and are referred to as measuring 'total' ADAb concentrations (i.e., drug-ADAb complexes as well as unbound ADAb). In practice, these drug-tolerant antibody assays are unlikely to be fully tolerant in the presence of high drug levels. The rate of ADAb detection is, therefore, highly dependent on the type of assay employed. However, based on the results of our survey, few clinicians (25%) are aware of the type of assay used to generate the results they interpret in their clinical practice (Samaan et al., 2018a).

An example of the potential difference in sensitivity between assays is evident in an early study describing the frequency of ADAb to adalimumab using a first-generation bridging ELISA, which reported a prevalence of 9.2% (Karmiris et al., 2009). However, when the same samples were re-analysed using an HMSA, the prevalence of antibodies more than doubled (Baert et al., 2016). Despite this difference in sensitivity, users should be reassured that clinically relevant high titre antibodies will be detectable using both assays. Low concentration ADAb detectable in a drug-tolerant assay, but 'hidden' with a drug-sensitive assay, may be transient and are usually not clinically relevant, at least in the short term (Cassinotti & Travis, 2009; Kopylov & Seidman, 2016; Kopylov et al., 2012; Pariente et al., 2012; Samaan et al., 2016; Van Stappen et al., 2018; Vande Casteele et al., 2012). There is, therefore, the potential to make incorrect short-term decisions based on this type of data, especially if the interpreting clinician is unaware of, or misunderstands the type of assay

used. This type of inappropriate action would have been taken by 55% of responders in our survey (Samaan et al., 2018a). However, it is possible that low titre antibodies might predict subsequent rising titres, sub-therapeutic levels and loss of response, suggesting that measuring total antibodies may offer the potential to intervene early to mitigate against the risk of immunogenicity. Overall, a detailed post-hoc analysis of the TAXIT study (Vande Casteele et al., 2015a) (which investigated the additional benefit of a drug-tolerant assay) concluded that although it allows closer follow-up of ADAb concentrations and identification of true transient versus persistent antibodies, it offers no clinical benefit to a drug-sensitive assay for the management of infliximab-treated patients in stable clinical remission (Van Stappen et al., 2018).

Despite variable analytical properties, a comparison of a bridging ELISA, RIA and HMSA found they provided overall similar guidance for clinical decision making and led to comparable outcomes in cases of infliximab treatment failure (Steenholdt et al., 2014). However, it should be noted that although the various commercially available ELISA kits are similarly priced in the UK, the price charged for carrying out an HMSA in some insurance-based health systems (e.g., the US) can be significantly more.

***Challenges in using therapeutic thresholds for drug and ADAb concentrations.***

The existence of an exposure-response relationship for both infliximab and adalimumab concentrations in UC and CD has been demonstrated by numerous studies (Papamichael & Cheifetz, 2016). However, universally applicable therapeutic thresholds have not yet been determined and due to the complex interplay of factors involved, defining these precisely may not be a realistic possibility. For example, it is understood that in the presence of active mucosal inflammation, levels may not necessarily be representative of tissue drug

concentrations as higher rates of drug clearance, due to increased tissue TNF burden and faecal loss, may result in lower serum concentrations (Brandse et al., 2015; Imaeda et al., 2014; Roblin et al., 2014; Roblin et al., 2015; Yarur et al., 2016b). Other factors, such as the desired outcome, may also require different thresholds (Papamichael et al., 2017). For example, endoscopic healing may require higher concentrations than symptomatic remission (Papamichael & Cheifetz, 2016) and fistula healing may require higher concentrations still (Yarur et al., 2017). These limitations notwithstanding, there is evidence suggesting that during maintenance therapy in CD, infliximab trough concentrations >3ug/ml are associated with significantly lower disease activity (as defined by CRP) (Jairath et al., 2016) and this cut-off has been evaluated elsewhere (Bortlik et al., 2013; Vande Casteele et al., 2015b). Similarly, for adalimumab, a cut-off of approximately 5 ug/ml has been independently described in two studies, which between them included endoscopic, clinical, and biochemical parameters in both UC and CD (Mazor et al., 2014; Roblin et al., 2014). Although these cut-offs have gained general acceptance as putative lower therapeutic thresholds (Mitrev et al., 2017), there currently remains a genuine paucity of prospective data to corroborate their use. This may explain why nearly a third of respondents to our questionnaire were unsure of what value to use as the lower therapeutic threshold for adalimumab and why 15% said they would dose intensify in the setting of secondary non-response despite a level of 10.3 ug/ml. There were, however, some responses with little or no rationale such as 7 or 9 ug/ml for infliximab, which were selected by a minority of our respondents (Samaan et al., 2018a).

Infliximab levels are conventionally measured at trough (just before the next infusion) and although this does not necessarily represent total drug exposure (Yamada et al., 2010), it is by far the most practical time point. However, where adalimumab is concerned, it is often

impractical to measure levels only at trough (e.g., when clinic appointments fall mid-cycle) and recent evidence suggests that mid-cycle levels are acceptable and can be interpreted with reasonable confidence (Ward et al., 2017). Nonetheless, only a third of our respondents reported practicing in this manner, with the remainder presumably preferring to request that patients return for blood tests at trough, a practice which may be considered unnecessary and inconvenient for patients.

Both the detection and quantification of ADAb is more complex and currently less well standardised than measurement of serum drug concentrations. The measures of quantification (units) used for individual assays may differ and are not readily comparable (Vande Casteele, 2017). The results are, therefore, assay-specific and the antibody titres (high, intermediate, or low) are often poorly defined, resulting in confusion amongst clinicians about their clinical significance. From an assay validation point of view, the US FDA issued their 'Guidance for Industry' in 2019, which made recommendations regarding estimation of the confirmatory assay cut-point. Their suggestion is to use an 80% to 90% one-sided lower confidence interval for the 99th percentile. The rationale being that as the purpose of this assay is to eliminate false-positive samples arising as a result of non-specific binding, it is adequate to use a 1% false-positive rate for the calculation of the confirmatory cut-point. Although prior attempts had also been made to standardise laboratory methodology (Gils et al., 2014), this remains a major challenge to the practical utility of anti-TNF TDM. Beyond the absolute values, the trend of ADAb quantities over serial measurements may be informative (Steenholdt et al., 1999), especially when assessing response to strategies intended to reduce immunogenicity, such as the addition of a concomitant immunomodulator (Ben-Horin et al., 2013), or to reduce the sequelae of immunogenicity, such as dose intensification (Yanai et al., 2015). It is important to

appreciate the difference and that dose escalation does not reduce immunogenicity per se; rather it temporarily overcomes the clinical problem caused by immunogenicity and 'hides' the positive titre, when measured using a drug-sensitive assay (Steenholdt et al., 2012; Van Stappen et al., 2018). However, it should be noted that other groups have described these strategies for the management of antibody positivity as 'prohibitively expensive' (Steenholdt et al., 1999) and 'biologically implausible' (Khanna et al., 2013).

## Golimumab

### Pre-clinical studies.

Golimumab (Simponi®, Janssen Biotech, Inc., Horsham, PA, USA) is the most recent anti-TNF agent to be approved for the treatment of moderate-to-severely active UC. Although it was approved for use in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis in 2009, it was not until 2013 that the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) granted approval for UC. Golimumab is a transgenic, fully human monoclonal immunoglobulin G1 antibody that is synthesised from TNF-immunised transgenic mice expressing human immunoglobulin G (Hutas, 2008; Lonberg, 2005). It differs from earlier anti-TNF agents in both its TNF binding affinity and protein stability (Lowenberg, de Boer, & Hoentjen, 2014). *In vitro* studies have demonstrated that golimumab binds to both bioactive forms of TNF (membrane-bound and soluble TNF) more avidly than infliximab or adalimumab (Shealy et al., 2010). This superior affinity has been shown to result in more potent neutralisation of TNF-induced cytotoxicity and endothelial cell activity. Subsequent *in vivo* studies (carried out in a murine model of TNF-mediated arthritis) have also suggested golimumab is more potent than infliximab with doses of 1 and

10 mg/kg significantly delaying disease progression, whereas infliximab was only effective at 10 mg/kg (Shealy et al., 2010). The excellent protein stability profile of golimumab is also relevant. This property means it can be prepared as a high concentration liquid formulation, making subcutaneous administration possible. This contrasts with infliximab which, owing to its inferior conformational stability, must be stored as a powder and reconstituted before being administered intravenously (Shealy et al., 2010).

**Induction treatment.**

Data to support golimumab's approval for induction of remission in UC were generated by the (Programme of Ulcerative colitis Research Studies Utilising an Investigational Treatment) PURSUIT-SC trial programme. This trial was a randomised, double-blind, placebo-controlled integrated phase 2 and 3 study designed to evaluate the safety and efficacy of subcutaneous golimumab for the induction of remission in moderate-to-severe UC (Sandborn et al., 2014b). In conjunction with this, an equivalent phase IV trial programme was commenced (PURSUIT-IV) assessing 2 and 4 mg/kg doses. However, as interim analysis suggested that induction regimens in the SC trial resulted in better clinical efficacy and PK profiles than in the phase IV trial, only the SC trial was taken forward.

Subjects enrolled to PURSUIT-SC were required to have failed or responded inadequately to standard therapy including oral 5-aminosalyciates, thiopurines, or oral corticosteroids. Patients who had previously been treated with anti-TNF therapy were excluded from taking part in the study. At least moderate disease activity was required defined as a Mayo score of 6 to 12 with an endoscopic subscore of 2 or more. Endoscopies were scored by the local investigator rather than being centrally read.

The initial phase 2 portion of the study was conducted to determine the dose-response relationship of subcutaneous golimumab. The data generated from this part of the study were then used to inform the design of the phase 3 portion of the trial, aimed to evaluate efficacy. In the phase 2 study, 169 patients were randomised to receive either placebo or one of three induction regimens: subcutaneous golimumab administered at weeks 0 and 2 in doses of 100/50 mg, 200/100 mg, or 400/200 mg. After safety, PK, and efficacy analyses, the 200/100 mg and 400/200 mg doses were selected for continuation in the phase 3 study.

Seven hundred and seventy-four patients were enrolled into the phase 3 portion of PURSUIT. The study's primary endpoint was clinical response at week 6 defined as a decrease in the Mayo score by at least 3 points and by 30% or more, with a bleeding subscore of 0 or 1, or decrease ≥1. Clinical remission was a secondary endpoint and was defined as a Mayo score ≤2 (with no subscore greater than 1). Additional secondary endpoints included rates of mucosal healing (MH) and impact on QoL. MH was defined as a Mayo endoscopic subscore of 0 or 1 and QoL was quantified using the Inflammatory Bowel Disease Questionnaire (IBDQ).

The study demonstrated positive findings for the primary and all secondary endpoints. A significantly larger proportion of subjects in the golimumab-treated groups achieved clinical response, clinical remission, MH, and had greater IBDQ scores when compared with placebo. Clinical response at week 6, the primary endpoint, was significantly greater in the 400/200 mg (55%) and 200/100 mg (51%) groups compared with placebo (30%, $p<0.0001$ for both treatment groups) as were MH rates (400/200 mg, 45%; 200/100 mg, 42%; placebo, 29%: $p<0.0001$ and $<0.0014$, respectively). In addition, although a Mayo score of 0 or 1 has been shown to be a clinically meaningful endpoint (Sandborn et al., 2009), as part

of the endoscopic evaluation, a more stringent endoscopic endpoint, Mayo 0 (normal mucosa or inactive disease) was also investigated. Whilst this endpoint was uncommon in participants at week 6, it occurred more commonly in golimumab-treated patients than those receiving placebo (12% in 400/200 mg regimen group vs. 4% in placebo group: $p<0.0001$). Clinical remission was also more common in golimumab-treated patients, approximately 18% of whom entered remission compared with only 6% of the placebo group ($p<0.0001$), resulting in a number needing treatment (approximately eight patients) (Hanauer, 2014). Biochemical evidence of improvement was also demonstrated with the mean (CRP concentration declining to a greater extent in the 400/200 mg and 200/100 mg groups compared with placebo; FC was not measured.

The authors of the PURSUIT-SC study concluded that subcutaneous golimumab induces clinical response, remission, and MH and improves QoL in patients with active UC. Based on these results, both the EMA and FDA approved the same induction regimen: 200 mg at week 0 and 100 mg at week 2, independent of weight.

However, subsequent comment on the trial outcome has suggested that although these endpoints are conventional – they parallel those used in trials of infliximab (Rutgeerts et al., 2005) and adalimumab (Sandborn et al.,2012) – and were achieved, they may not tell clinicians all they need to know about the drug. For example, although golimumab is superior to placebo, it remains true that the vast majority of patients who respond to the drug are still symptomatic, on concomitant steroids, and without a ‘normal or inactive’ mucosal appearance (Hanauer, 2014) See Figure 2 and Figure 3.

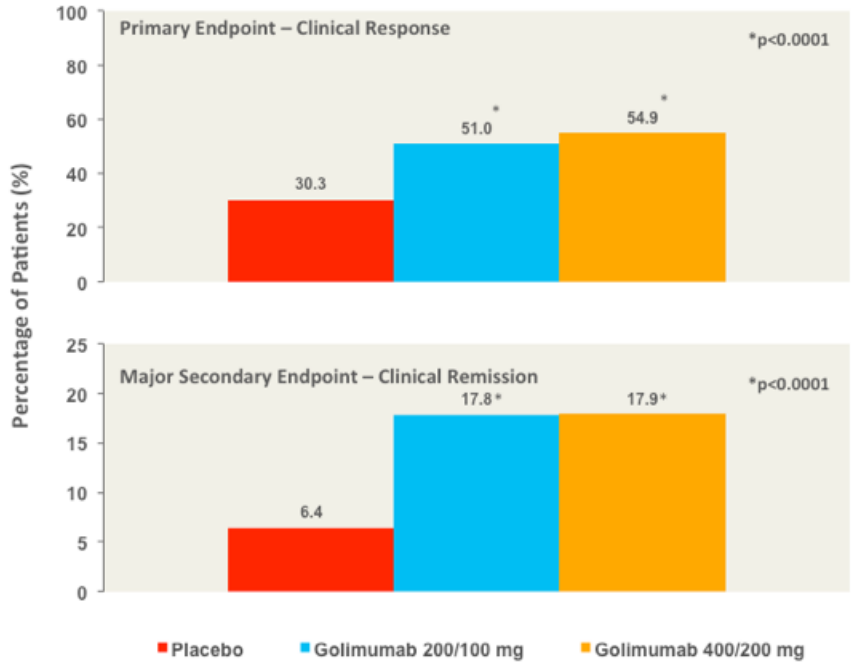



*Figure 2.* Primary and major secondary endpoints in PURSUIT-SC

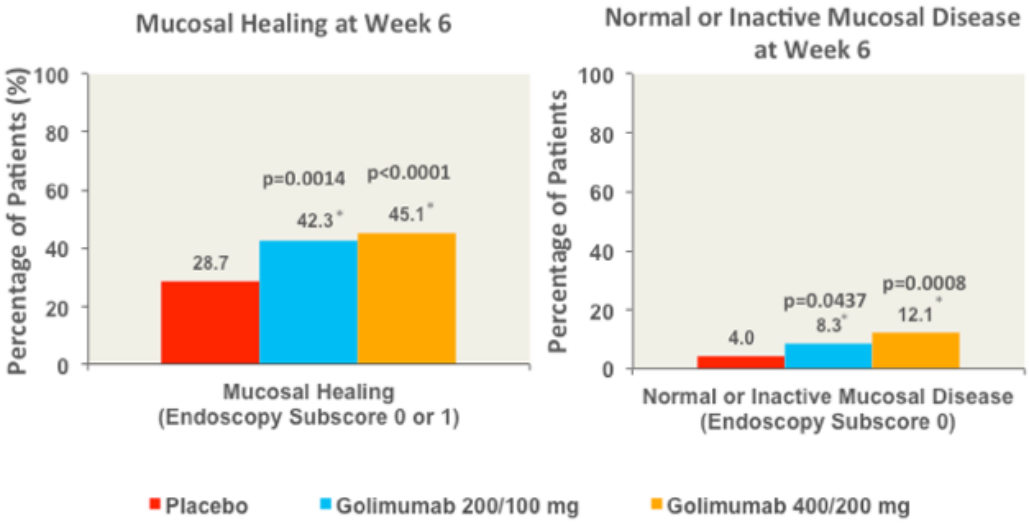



*Figure 3*. Week 6 endoscopic outcomes in PURSUIT-SC

**Maintenance treatment.**

All subjects from the PURSUIT-SC trial programme were eligible for enrolment into the PURSUIT-Maintenance (PURSUIT-M) study (Sandborn et al., 2014a). The 464 patients who

achieved a clinical response with golimumab induction therapy were subsequently randomised to either placebo or treatment with 50 mg or 100 mg of golimumab administered every 4 weeks. A further 129 patients who had responded to placebo continued on placebo maintenance therapy, and 635 patients who did not respond (to either placebo or golimumab) received open-label golimumab 100 mg every 4 weeks. The primary endpoint was clinical response maintained through to week 54. To demonstrate maintained response, patients were assessed using the partial Mayo score at 4-weekly intervals with the addition of the endoscopic component (to generate the full Mayo score) at weeks 30 and 54. Patients who met predefined criteria for a clinical flare at any time point underwent an endoscopy to confirm loss of response (see Figure 4).

Golimumab was shown to maintain response in 47% and 50% of patients who received 50 mg or 100 mg golimumab every 4 weeks, respectively, versus 31% in the placebo group ($p=0.010$ and $p<0.001$, respectively) thus, meeting the trial's primary endpoint.

The secondary endpoint of clinical remission at both weeks 30 and 54 was achieved by 16%, 23%, and 28% in the placebo, golimumab 50 mg, and golimumab 100 mg groups, respectively. This difference reached statistical significance in the 100 mg group but not in the 50 mg group, despite a numerical advantage being seen ($p=0.122$ and $p=0.004$ for 50 mg and 100 mg golimumab-treated patients versus placebo). Additional secondary endpoints of MH, and corticosteroid-free remission by week 54 were also significantly more likely to occur in patients treated with golimumab compared with placebo (see Figure 5 and Figure 6.)

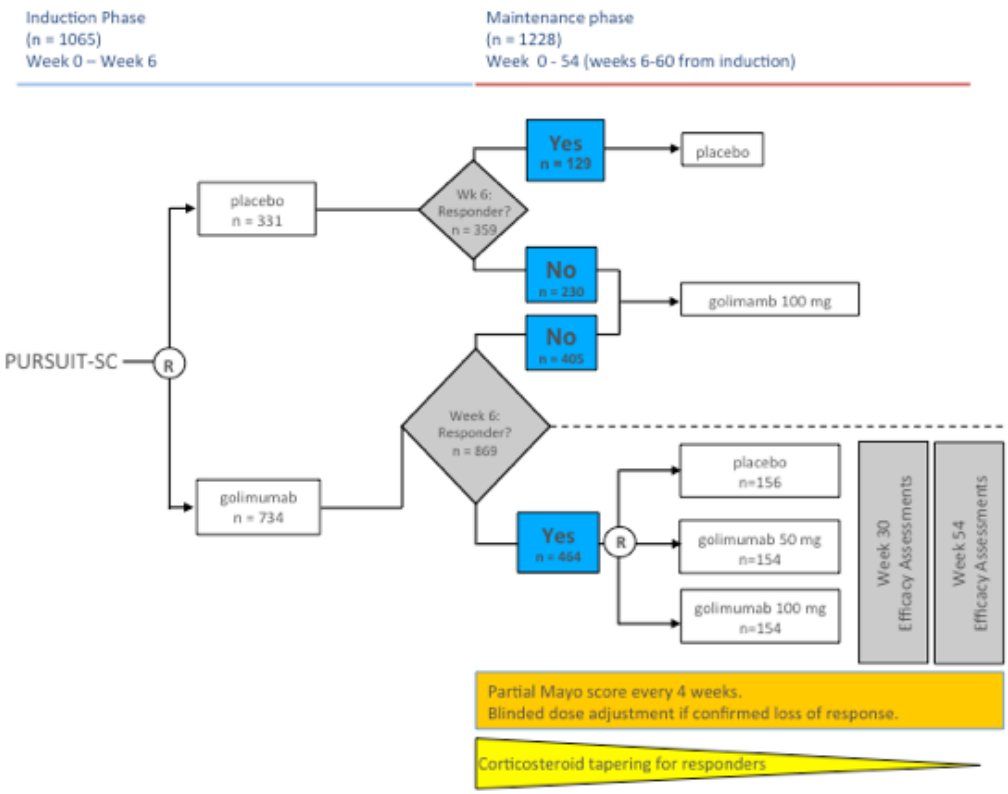



*Figure 4.* Overview of the study design of the PURSUIT program (R, randomisation)

Based on the results of PURSUIT-M, golimumab was approved by both the EMA and FDA. However, the dosing regimen approved by each differs slightly. In the US, all patients receive 100 mg every 4 weeks, whilst in Europe patients with weight below 80 kg receive 50 mg every 4 weeks and only those with weight over 80 kg receive 100 mg every 4 weeks.

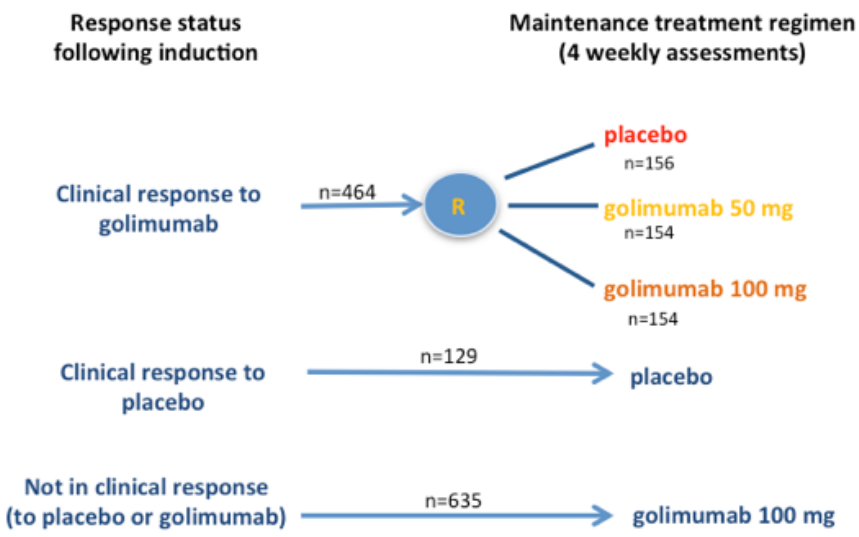



*Figure 5.* Patient flow between induction (PURSUIT-SC) and maintenance (PURSUIT-M) phases (R, randomisation)

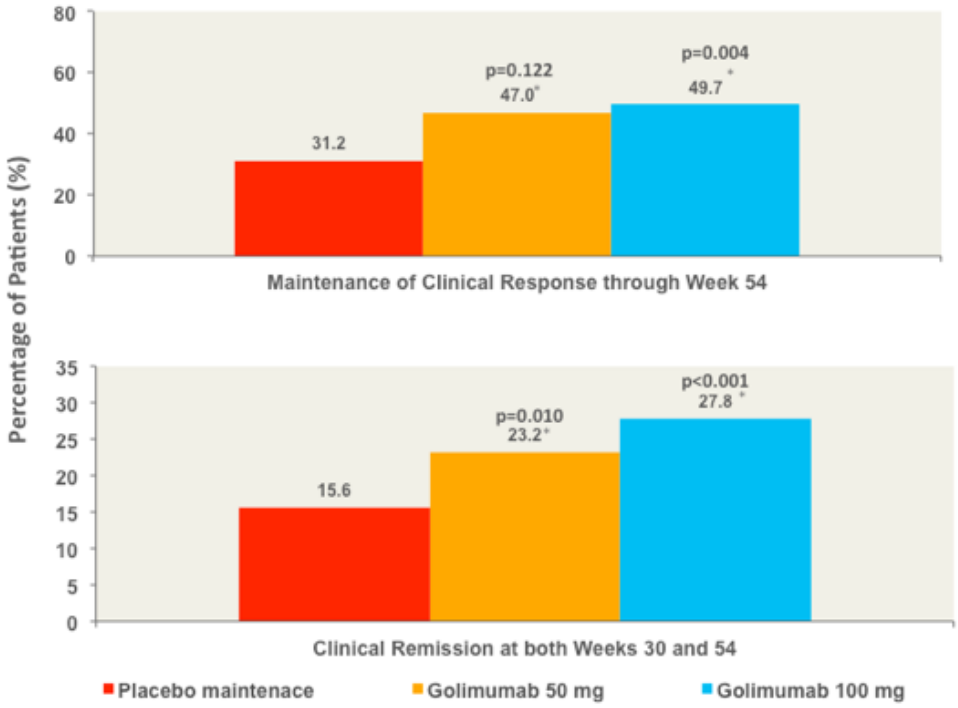



*Figure 6.* Proportion of golimumab induction responders who maintained clinical response through week 54 (above) achieved clinical remission at both weeks 30 and 54 (below)

The design of PURSUIT-M was novel in several ways. Firstly, its definition of maintained response was more stringent than any previously seen in a UC trial. Long-term continuous efficacy was evaluated over the course of 15 prospective assessments without loss of response permitted at any time point. This compares with three assessments undertaken as part of the ACT-1 (Rutgeerts et al., 2005) maintenance trial or the two seen in the ACT-2 (Rutgeerts et al., 2005) and ULTRA (Sandborn et al., 2012) maintenance trials. Second, PURSUIT-M was the first randomised withdrawal study of an anti-TNF in UC, thus clarifying that induction only is insufficient to maintain a long-term response (see Figure 7).

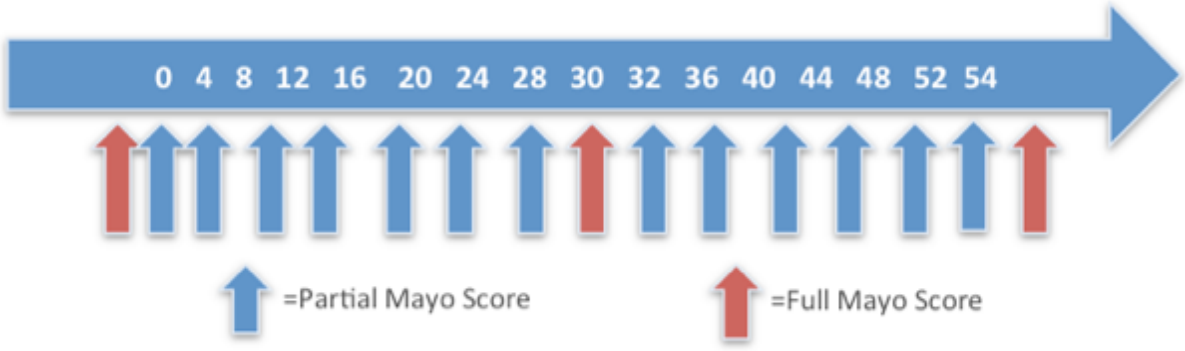



*Figure 7.* Diagram demonstrating distribution of the fifteen clinical assessments made as part of PURSUIT

More recently, the long-term extension (LTE) of the PURSUIT-M trial (Solberg et al., 2009) has been published. The LTE included 666 patients who were responders and completed treatment through to week 52, who were then followed to assess safety and efficacy for an additional three years. Efficacy analyses were performed on 195 of these patients, that is, those that were randomised to golimumab maintenance at baseline and continued to take the drug during the LTE. Of these patients, 134 remained on golimumab until week 216 and 77.6% of these patients had a Physicians' Global Assessment score of 0 at that time point equating to 53.3%, if an intention-to-treat analysis was used.

**Safety.**

It remains too early for any safety registry data for golimumab in IBD to mirror the results from the infliximab (TREAT: Lichtenstein et al., 2012) and adalimumab (PYRAMID: D'Haens et al., 2011) registries. However, safety analyses from the PURSUIT trials and results as well as long-term extensions of RCTs carried out in rheumatoid diseases help to inform this area (Kay et al., 2015). During the PURSUIT trial programme, the observed safety signals were reassuring and consistent with experience gained from use in rheumatoid arthritis as well as with the safety profile of the other anti-TNF agents. Four cases of tuberculosis were seen, all in golimumab-treated patients (who were also receiving corticosteroids) living in endemic regions, with one resulting death. This finding should serve to underscore the importance of robust pre-treatment screening for tuberculosis in clinical practice. Overall, the percentage of patients with adverse events were similar across the golimumab treatment groups but were somewhat higher compared with the placebo group. However, when the safety data were normalised to 100 years of patient follow-up, the incidence of adverse events was comparable across each of the treatment groups (see Table 3). The most commonly observed adverse events (other than UC flare) were nasopharyngitis, headache, and arthralgia. Injection site reactions were more common in golimumab-treated patients and occurred in 7.1% of patients receiving 100 mg golimumab, 1.9% receiving 50 mg golimumab and 1.9% receiving placebo. Other than this finding, no significant dose-dependent accumulation of adverse events was seen.

In a 3-year follow-up of 2226 patients with rheumatological conditions (rheumatoid arthritis, psoriatic arthritis, or ankylosing spondylitis) treated with golimumab in clinical trials, it was observed that golimumab 100 mg showed numerically higher incidences of

serious infections, demyelinating events and lymphoma than 50 mg (Kay et al., 2015). Although none of these differences reached statistical significance, further longitudinal safety data are yet to be reported at 5 years to clarify further the relationship with these potential long-term adverse effects.

**Table 3**
*Key safety findings, normalised to 100 patient-years of follow-up to week 54*

| | **Placebo** (n=156) | **50 mg** (n=154) | **100 mg** (n=154) |
|---|---|---|---|
| | **Number of specified events per 100-patient years of follow-up** | | |
| Adverse event | 211 | 187 | 173 |
| Serious adverse events | 13 | 10 | 17 |
| Infections | 55 | 61 | 60 |
| Serious infections | 3 | 4 | 4 |
| Adverse Events leading to discontinuation of study agent | 10 | 6 | 10 |

**The position of golimumab in the UC therapeutic algorithm.**

Where golimumab is positioned in the treatment of moderate-to-severe UC will depend on a number of factors, some of which will vary across individual healthcare systems, centres, clinicians, and patients. Its pattern and frequency of use will, therefore, likely vary. No head-to-head trials have been performed comparing different anti-TNF agents in UC. However, a network meta-analysis, comprising 2,282 patients receiving anti-TNF (infliximab, adalimumab, or golimumab) or vedolizumab for UC (Danese et al., 2014) has been published. This identified a trend suggesting that infliximab may be slightly more effective

than the other biologic agents although the only statistically significant difference was seen when comparing the ability of infliximab and adalimumab in inducing clinical response (odds ratio 2.36; confidence interval 1.22–4.63) and MH (odds ratio 2.0; confidence interval 1.13–3.59). It is important to remember the weaknesses of comparing results from different trials; however golimumab and adalimumab seem to be approximately equivalent in terms of efficacy and safety. Head-to-head RCT data exist to compare vedolizumab to adalimumab in UC and demonstrate the superiority of vedolizumab with respect to achievement of clinical remission and endoscopic improvement, but not corticosteroid-free clinical remission (Sands et al., 2019a). One may well, therefore, expect a similar order of efficacy if golimumab were compared to vedolizumab.

The use of golimumab as a rescue therapy in acute severe hospitalised patients with UC has not been studied and infliximab will remain the anti-TNF agent of choice for these patients. Golimumab has also not been evaluated formally in patients who have failed other anti-TNF agents. Although observation data exist to suggest effectiveness in this setting (Taxonera et al., 2017), in the context of mechanistic failure (i.e., non-response in the presence of adequate anti-TNF levels), a switch out of class would seem preferable. Finally, whether vedolizumab, ustekinumab, or tofacitinib may eventually become the preferred advanced therapy for UC currently remains unclear.

**Real-world observational effectiveness studies.**

As with all new drugs, real-world data publications allow an assessment of the use of the drug in everyday practice and, therefore, complement the data derived from clinical trials. Whilst the quality of the data is unarguably poorer, the patients who are included in such observational studies are more representative of ‘real-world’ practice than patients who

participate in clinical trials, who are, by definition, a well-defined subsection of the overall patient cohort.

Detrez et al. (2016) reported a cohort of 21 patients from Belgium treated with golimumab for moderate-to-severe UC. Just under half of the patients (48%) achieved partial clinical response at week 14, defined as marked clinical improvement. Complete clinical response was only achieved in 14% of patients, while only 19% of patients achieved MH. However, in contrast to the PURSUIT trials, in which all participants were anti-TNF naïve, 52% were previously exposed to anti-TNF in this cohort.

Bosca-Watts et al., (2016) prospectively followed 33 patients with moderate-to-severe UC commenced on golimumab across several centres in Spain. Most of these patients (73%) were anti-TNF exposed. Despite this, clinical response (defined by a decrease in the partial Mayo score of at least 3 points) was achieved by 70% of patients at week 14, and 51.5% achieved clinical remission. MH data were not reported, but the mean FC value fell from 300 µg/g to 170.5 µg/g.

Taxonera et al. (2017), performed a retrospective analysis on 142 patients with UC treated with golimumab across several Spanish centres. Again, most of these patients (60%) had been previously exposed to anti-TNF. Short-term clinical response, defined as a 3-point decrease in the partial Mayo score or a decrease of ≥50% in the partial Mayo score and a final partial Mayo score of ≤2 at 8 weeks, was seen in 64.8% of the patients. Short-term clinical remission rate was 31.7% and both clinical response and remission rates were lower if golimumab was given as the third anti-TNF agent. However, after a median follow-up of 12 months, 42% of patients had golimumab failure, the majority of which was due to primary non-response.

Tursi et al. (2017) prospectively observed 93 patients over a 6-month period, the majority of whom were anti-TNF naïve (88.8%). At 6 months, clinical remission, defined as a Mayo score ≤2, was achieved by 36.5% patients, and 64.5% achieved clinical response. However, only 19% had steroid-free remission at week 26 with the same number achieving MH.

Bossuyt et al. (2019) retrospectively analysed 91 patients who were previously included in the SMART study (an open-label observational study that explored patient preference for either pen or syringe to deliver golimumab in Belgium). The majority of these patients (87%) were anti-TNF naïve, and all received standard induction and maintenance regimens with the option of dose optimisation during the maintenance phase. The primary endpoint was golimumab continuation without steroids at week 26, which occurred in 41% of patients. At week 52 this reduced to 30%. Thirty-four percent of patients had primary non-response and 23% had secondary loss of response within the first year. The MH rate at week 14 was 40%, and if this outcome was achieved it predicted steroid-free golimumab continuation at week 52 (OR 9.38, p<0,001).

Probert et al. (2018) prospectively analysed 205 anti-TNF naïve patients as a part of the UK-based GO-COLITIS trial. The primary endpoint was sustained clinical response through to week 54 as defined by a decrease in the partial Mayo score (PMS) of ≥2 points and ≥30% from baseline, plus either a decrease in the rectal bleeding subscore of ≥1 point or an absolute rectal bleeding subscore of 0 or 1. This was achieved in only 25% of patients. Interestingly, of the 52 patients that achieved clinical response at week 54, a significant proportion (60%) discontinued therapy but still maintained clinical response for a further 12 weeks.

Finally, O'Connell et al. (2018) evaluated 72 UC patients receiving golimumab in Ireland. Clinical response was measured at 3 months and corticosteroid-free remission was measured at 6 months, the rates being 55% and 39% respectively. Over a mean follow-up duration of 8.7 months (0.4–39.2), 44% patients discontinued the drug. See Table 4.

**Table 4**
*Summary of real-world observational studies of golimumab in UC*

| Study | Year | Number of patients | Anti-TNF exposed | Follow-up period | Clinical response | Clinical remission | MH |
|---|---|---|---|---|---|---|---|
| **Detrez et al. (2016)** | 2016 | 21 | 52% | 14 weeks | 14% | - | 19% |
| **Bosca-Watts et al. (2016)** | 2016 | 33 | 73% | 14 weeks | 70% | 52% | - |
| **Taxonera et al. (2017)** | 2017 | 142 | 60% | 8 weeks | 65% | 32% | - |
| **Tursi et al. (2017)** | 2017 | 93 | 11% | 24 weeks | 65% | 37% | 19% |
| **Probert et al. (2018)** | 2018 | 205 | 0% | 6 weeks | 69% | 39% | - |
| | | | | 54 weeks | 25% | 18% | - |
| **O'Connell et al. (2018)** | 2018 | 72 | 36% | 12 weeks | 55% | - | - |
| **Bossuyt et al. (2019)** | 2019 | 87 | 13% | 14 weeks | - | - | 40% |

### Use in CD.

Despite it not being licensed currently there is recent evidence to suggest that golimumab is also effective in the treatment of CD. A retrospective analysis of 115 patients, the majority of which had already lost response to at least one anti-TNF therapy, observed a clinical response of 55.8% after a mean duration of 3.8 months although most patients (80.7%) required dose escalation by 24 months (Martineau et al., 2017). A further retrospective observational study of 45 patients reported a clinical response rate of 77.7% at 3 months (Greener et al., 2018). However, induction and maintenance regimens were higher than the current regimens used to treat UC.

### Pharmacokinetics.

#### *Serum concentrations and exposure-response relationship.*

The initial studies carried out to investigate golimumab serum drug levels and ADAb took place in the context of its use in rheumatoid arthritis (Kneepkens et al., 2014). In a prospective, observational cohort study consisting of 37 patients, a similar correlation between TL quartile and response was observed as described above. The lowest quartile (golimumab <0.25 mg/L) comprised 32% of all non-responders, whilst the highest (golimumab >1.4 mg/L) comprised 47% of all responders. Three patients were found to have high ADAb titres. These resulted in undetectable golimumab levels and poor clinical outcome. ADAb were also detected in a small minority of patients (2.9%) in the PURSUIT trials. Their occurrence was less common in patients who were receiving concomitant immunomodulators (1.5%) compared with patients who were not (3.5%) (Adedokun et al., 2017).

Analysis of serum golimumab concentrations (SGC) during PURSUIT was carried out using a validated assay and revealed that serum concentrations were dose proportional. Furthermore, there was an exposure-response relationship: those with higher serum concentrations of golimumab had higher rates of response and remission as well as greater improvement in median composite Mayo scores. The median TL serum concentration (measured prior to administrations at weeks 8, 12, 20, 28, 36 and 44) was 0.69 – 0.83 ug/mL in the golimumab 50 mg group and 1.33 – 1.58 ug/mL in the golimumab 100 mg group. Steady-state PK was achieved after approximately 8 weeks of maintenance treatment with no carry-over effect observed from the induction dose regimen received. Further PK analysis demonstrated that the bioavailability of golimumab is approximately 52% and that its half-life is approximately 10.5 days. These values compare with 64% and approximately 14 days for adalimumab (Mease, 2007).

In PURSUIT-SC, the change from baseline Mayo score and rates of clinical response and clinical remission at week 6 increased with increasing quartiles of serum golimumab concentration. In the subsequent maintenance trial, a combined analysis of patients randomised to golimumab 50 mg and 100 mg groups showed that more patients in the higher serum golimumab concentration quartiles achieved clinical response through to week 54, or clinical remission at both weeks 30 and 54, when compared with those in the lower serum concentration quartiles. This raises the possibility that dose escalation could be an effective strategy for patients with lower drug levels, although it should be noted that there was no difference in the rate of clinical response in secondary non-responders who received dose escalation, compared to those who maintained the 50 mg dose, albeit only in a small number of patients (Hanauer, 2014).

An exposure-response relationship was also observed in a post-hoc analysis in PURSUIT. As part of this detailed analysis, using receiver operating characteristic (ROC) curve analysis, the authors identified a level of 2.5 µg/mL at week 6 and 1.4 µg/mL during maintenance as being desirable concentration targets for attainment of optimal clinical outcomes. (Adedokun et al., 2017) (See Figure 8 and Figure 9.)

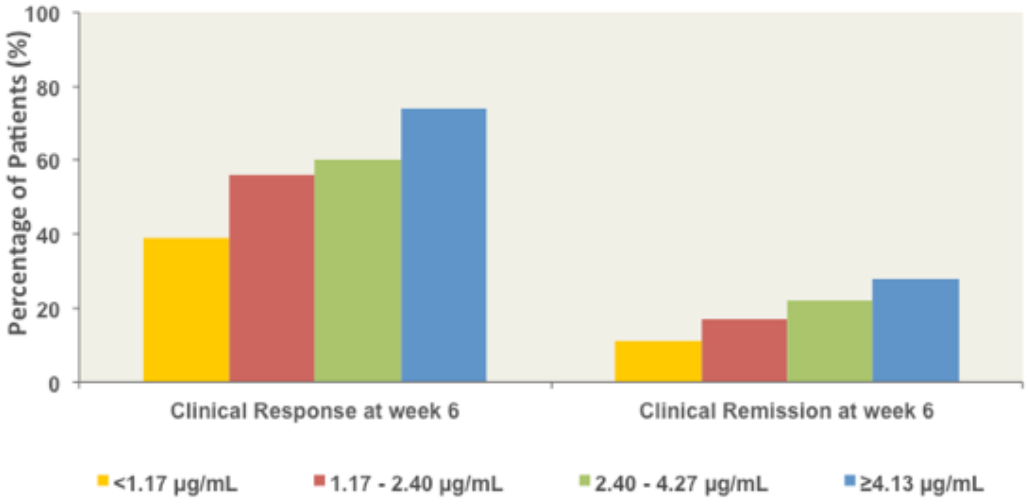



*Figure 8.* Proportion of patients achieving clinical response and remission by serum golimumab concentration at week 6 in PURSUIT-SC

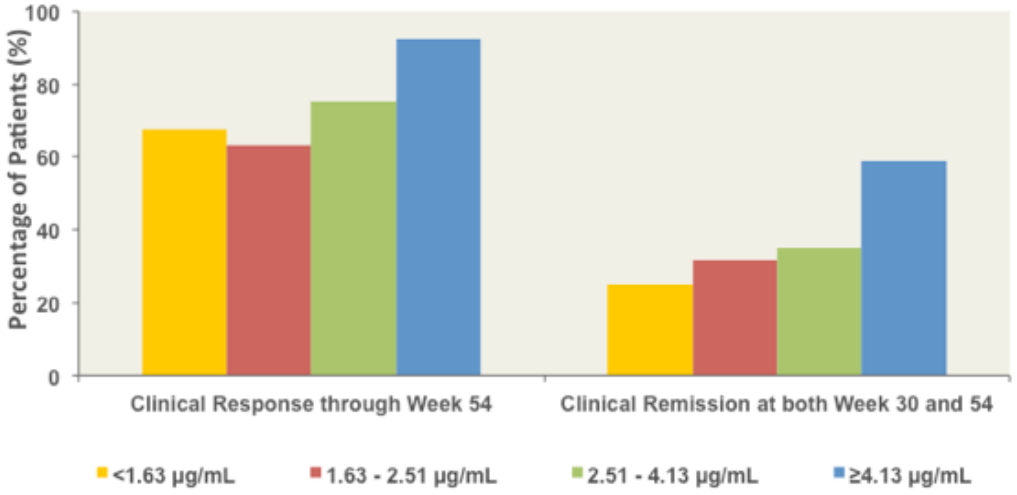



*Figure 9.* Proportion of patients with clinical response through to week 54 (left) or in clinical remission at both weeks 30 and 54 (right) by serum golimumab concentration quartiles at week 54 in PURSUIT-M

Detrez et al. (2016) also collected serum drug levels from patients during the first 14 weeks of therapy in a small observational cohort study (n= 21). In this study, patients who achieved partial response had higher drug levels at weeks 2 and 6 compared with non-responders; 10.0 µg/mL versus 7.4 µg/mL and 5.1 µg/mL versus 2.1 µg/mL, respectively. These levels were much higher than those in the PURSUIT-SC post-hoc analysis but the majority of patients in the former study had already been exposed to anti-TNF therapy.

During the course of 2019 there have been a series of publications reporting trials designed to investigate the PK of golimumab and define optimal thresholds for use in TDM algorithms. These studies employed a range of methods and reported on potential golimumab target concentrations across various time points and endpoints (summarised in Table 5). The first of these to publish was a study carried out by the Portuguese IBD group (Grupo de Estudo da Doença Inflamatória Intestinal, GEDII) and involved an exploratory, pre-specified sub-analysis of samples from patients recruited to the EVOLUTION study (Magro et al., 2019a). EVOLUTION was an exploratory, multicentre, open-label, prospective, interventional, single-arm study, conducted across nine Portuguese centres. It aimed to evaluate the utility of soluble suppressor of tumourigenicity 2 as a surrogate biomarker of disease outcome and therapeutic response, in moderate-to-severe UC patients treated with golimumab. As part of the study, 34 patients were assessed clinically, biochemically, endoscopically and histologically at weeks 6 and 16 after commencing golimumab and also had week 6 serum golimumab TLs measured. Overall, 47.1% and 14.7% of patients achieved clinical response and remission with significantly higher serum golimumab TLs in patients with early response or remission (3.7 µg/mL vs, 1.3 µg/mL, $p$=0.0013; and 3.1 µg/mL vs. 1.7 µg/mL, $p$=0.0164, respectively). In addition, golimumab TLs were significantly higher in

patients achieving histological remission (4.2 µg/mL vs. 1.7 µg/mL, *p*=0.0049). Week 6 golimumab TLs were inversely correlated with the total Mayo score (rs = -0.546; *p*=0.0008), the Mayo endoscopic subscore (rs = -0.381; *p*=0.0262), the Geboes histological activity score (rs = -0.464; *p*=0.0057), and FC levels (rs = -0.497; *p*=0.0044) (Magro et al., 2019b). The authors, therefore, concluded that greater exposure during the induction phase is associated with objective response. However, they stopped short of carrying out ROC analysis to suggest a putative therapeutic threshold. They also found no association between week 6 golimumab TL and clinical, biochemical, endoscopic, or histologic outcomes at week 16, suggesting that early exposure may not influence short-term maintenance.

More recently, a study conducted between KU Leuven and a centre in Slovenia, reported on a population PK and exposure-response model for targeting endoscopic remission in UC (Dreesen et al., 2019). Dreesen et al. (2019) included a total of 56 patients across three study cohorts, a prospective group (also included in a separate study investigating golimumab dried blood spot analysis, GOUDA: Detrez et al., 2018) as well as retrospective groups at each of the study sites. The study involved over 700 samples – either from venepuncture or capillary puncture (finger prick) dried blood spots – across multiple time points to facilitate the generation of a Markov model. They found that golimumab PK was best described using a 2-compartment model with linear (first-order) absorption and elimination. In addition, they identified the development of antibodies to golimumab and previous biological therapy as factors that reduced golimumab exposure (Dreesen et al., 2019). Despite the fact that interindividual PK variability remained largely unexplained, their Markov model allowed the identification of golimumab trough thresholds that associate with endoscopic remission (Mayo endoscopic subscore 0 or 1). Thresholds of 7.4 ug/mL at

week 6 and 3.2 ug/mL at week 14 predicted endoscopic remission at week 14 (positive predictive values 83% and 91%, negative predictive values 82% and 67%, respectively). The 3.2 ug/mL week 6 target predicted 38% and 44% chances of achieving endoscopic remission in patients with a baseline Mayo endoscopic score of 3 and 2, respectively (Dreesen et al., 2019).

Most recently, a Canadian group published a retrospective study of 58 IBD patients (39 with CD and 19 with UC/IBDU) who were assessed for MH and golimumab TL at a median of 44 weeks from treatment initiation. MH was defined here as Mayo ≤1 in UC/IBDU, simple endoscopic score for Crohn's disease (SES-CD) 0 to 5 in CD, normal or quiescent disease on radiology reports, or FC <250 μg/mL. Amongst the CD cohort, ROC curve analysis demonstrated that a TL >8 μg/mL during maintenance therapy was associated with MH (sensitivity 67%, specificity 88%, area under the curve [AUC] 0.76) (Boland et al., 2019). However, a robust threshold was more difficult to define for the UC/IBDU cohort, perhaps due to the small number of patients. Although the difference was not statistically significant, there was a trend for patients with MH to have higher TLs (4.8 ug/mL) than those without (2.05 ug/mL). A threshold of 5.6 ug/mL was suggested for MH but in view of the lack of statistical power, the authors recommended exercising caution when interpreting this finding (Boland et al., 2019). As part of this, the effect of combination therapy on serum golimumab levels was also investigated. Twenty-three (40%) of the cohort were on a concomitant immunosuppressant (either thiopurine or methotrexate) at the time of evaluation and no significant difference was found between the groups (Boland et al., 2019).

**Table 5**

*Summary of golimumab therapeutic thresholds described for various outcomes*

| Study | Year | n | Time point | Endpoint | Threshold (ug/ml) |
|---|---|---|---|---|---|
| **Detrez et al. (2016)** | 2016 | 21 | Week 14 | Partial clinical response | 2.6 |
| **Adedokun et al. (2017)** | 2017 | 1064 | Week 6 | Clinical response | 2.5 |
| | | 464 | Week 44 | Clinical remission | 1.4 |
| **Dreesen et al. (2019)** | 2019 | 56 | Week 6 | Endoscopic remission | 7.4 |
| | | | Week 14 | | 3.2 |
| **Boland et al. (2019)** | 2019 | 19 | Week 44 (median) | Endoscopic remission | 5.6 |
| **Magro et al. (2019b)** | 2019 | 34 | Week 6 | Clinical, biochemical, endoscopic, and histologic | Not stated |

In 2017, the American Gastroenterological Association published a guideline regarding the TDM in IBD (Feuerstein et al., 2017). As part of this evidence-based, expert consensus exercise, they made recommendations for suggested trough concentration targets for infliximab, adalimumab, and certolizumab. However, as this guideline predated the evidence described above, the group concluded that there was insufficient data to give any specific recommendation for a golimumab threshold. This recommendation was in keeping with the concurrently published consensus from the American Gastroenterological Association technical review of the role of TDM in IBD (Vande Casteele et al., 2017).

***Immunogenicity.***

Antibodies to golimumab were originally considered to be a rare phenomenon, leading to the premise that the drug had low immunogenic potential. This understanding was based upon data from the PURSUIT trial programme (Sandborn et al., 2014a; Sandborn et al., 2014b) and its subsequent PK sub-analysis (Adedokun et al., 2017), which described antibodies in very low numbers. These analyses were originally carried out using a drug-sensitive ELISA developed by Janssen. Other studies, carried out subsequently but using similarly drug-sensitive assays, have shown correspondingly low rates of antibody detection, some even showing that no patients developed detectable antibodies. This assumption of limited immunogenicity has, however, more recently been demonstrated to, in fact, be an underestimation. Detrez et al. (2016) were the first to describe the development of a drug-tolerant golimumab antibody ELISA and demonstrated that the rate of antibody detection increased from 0/21 patients using the drug-sensitive assay, to 4/21 (19%) with the drug-tolerant method (Detrez et al., 2016). Similarly, re-analysis of the PURSUIT samples with a drug-tolerant assay showed a tenfold increase from 2.8% of patients to 21.8% testing positive for antibodies at some point during the 54 week maintenance trial (Adedokun et al., 2019). Whether a drug-sensitive or drug-tolerant assay is employed, combination therapy has been shown to reduce the rate of antibody formation. However, this difference is much more marked when using a drug-tolerant assay (Adedokun et al., 2017; Adedokun et al., 2019).

Although antibodies to golimumab have been repeatedly shown to be a key determinant of its PK and (perhaps to a lesser degree) its efficacy, the exact impact of the additional antibodies detected using a drug-tolerant assay (i.e., in the presence of circulating drug) is

not yet fully appreciated. Adedokun et al.'s (2019) analyses suggested that antibodies picked up by the drug-sensitive assay (i.e., in the absence of detectable drug) are likely to have a more significant deleterious effect on UC outcomes than those found using only the tolerant assay. The same finding was replicated in a cohort of patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis (Leu et al., 2019) (see Table 6).

**Table 6**

*Summary of golimumab antidrug antibody detection with various assays (RA, rheumatoid arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis)*

| Study | Year | n | Assay | Drug sensitivity | Proportion of patients with antidrug antibodies (%) |
|---|---|---|---|---|---|
| **Detrez et al. (2016)** | 2016 | 21 | ELISA | Sensitive | 0 |
| | | | | Tolerant | 19 |
| **Adedokun et al. (2017)** | 2017 | 1528 | ELISA | Sensitive | 2.9 |
| **Dreesen et al. (2019)** | 2019 | 56 | ELISA | Tolerant | 13 |
| **Boland et al. (2019)** | 2019 | 19 | ELISA | Sensitive | 1.7 |
| **Magro et al. (2019b)** | 2019 | 34 | ELISA | Sensitive | 0 |
| **Adedokun et al. (2019)** | 2019 | 1195 | ELISA | Sensitive | 2.8 |
| **(UC)** | | | | Tolerant | 21.8 |
| | 2019 | 1109 | ELISA | Sensitive | 4.1 |

| Study | Year | n | Assay | Drug sensitivity | Proportion of patients with antidrug antibodies (%) |
|---|---|---|---|---|---|
| **Adedokun et al. (2019)** **(RA, PsA, AS)** | | | | Tolerant | 31.7 |
| **Berger et al. (2019)** | 2019 | 78 | ELISA & RIA | Sensitive | 2.6 |
| | | | | Tolerant | 28.2 |
| **Detrez et al. (2018)** | 2019 | 10 | ELISA | Tolerant | 30 |

***Dose flexibility.***

The original dosing schedule for golimumab was restrictive and included no protocolised or licensed escalation. However, in some published series, dose escalation has been described and there are data that it can recapture response. Taxonera et al. (2017) reported 28 patients who were dose escalated either by increasing the dose from 50 to 100 mg 4-weekly (90.3%), from 100 to 200 mg 4-weekly (3.2%) or to 100 mg every 2 weeks (6.4%). A significant number (71%) of patients were able to recapture response with this strategy (Taxonera et al., 2017). In Ireland presently, the randomised multi-centred GOAL-ARC study is progressively dose-escalating patients (up to 200 mg 4-weekly) based on FC and drug levels (Sheridan et al., 2018). The results of this study should offer evidence regarding how best to modify maintenance dosing to optimise outcomes.

On the basis of emerging evidence, there has recently been a change in the golimumab induction dosing approved by the EMA. This allows patients with body weight <80 kg who

have an inadequate response to induction dosing at weeks 0 and 2, to continue with 100 mg at week 6 and every 4 weeks thereafter, instead of 50mg. The change took into account the type of data generated by Philip et al. (2019) in their post-hoc analysis of data from PURSUIT-M. They found that early use of the 100 mg maintenance dose led to achievement of clinical response at week 14 in 28% of patients who had failed to respond to golimumab at week 6. Early non-responders <80 kg who received the 100 mg maintenance dose were also found to have achieved adequate golimumab concentrations (Philip et al., 2019).

The overall aim of this thesis is to provide new insights into the potential role of TDM in maximising the benefit that golimumab can provide for patients with UC. The first steps in this process are to consider the range of available techniques used to measure golimumab and anti-golimumab antibody concentrations, and to evaluate the operating characteristics of a commercially available assay through an assay validation/verification program. The following chapter will address the real-world effectiveness of golimumab through a retrospective, observational study and will provide information regarding optimisation strategies such as empirical dose escalation. Finally, by designing and performing a prospective, phase IV clinical trial we aim to gain deeper insights into important pharmacokinetic aspects of golimumabs use, including therapeutic thresholds during the induction and maintenance phases of treatment.

# Chapter 2: Measurement of Serum Golimumab and Anti-golimumab Antibody Concentrations

It is well recognised that protein-based drugs, such as golimumab, exhibit interindividual and intra-individual variability in drug PK and PD. One major factor attributing to this variability is the formation of ADAb in a subset of patients, irrespective of disease indication, or degree of humanisation of the antibody. Measuring ADAb and monitoring of drug PK are essential for drug development. TDM with the measurement of drug and ADAb in serum is also a well-established tool in the use of older anti-TNF agents, such as adalimumab and infliximab, where it can rationalise treatment decisions at the time of loss of response and help to optimise dosing in patients on maintenance therapy.

Many assays and different assay formats exist to measure drug and ADAb in serum. The longest-standing and most commonly employed technique for measuring drug and ADAb concentrations is by using an ELISA. Alternative techniques include RIA, HMSA, and more recently chemiluminescence (CLIA) and electrochemiluminescence immunoassays (ECLIA) have been developed. Although there is no gold-standard technique, ELISA has the benefit of being relatively simple and inexpensive.

## Aims

The aims of this chapter were, firstly to review the available assays and corresponding data for the measurement of golimumab and anti-golimumab antibody concentrations and, secondly to conduct a laboratory verification exercise to better understand the operating characteristics of the commercially available assay used in the Viapath laboratory at Guy's & St Thomas'.

## Enzyme-linked Immunosorbent Assays

To measure drug concentrations using an ELISA, microwells are coated with either a mouse monoclonal ADAb or TNF to capture the TNF antagonist (in this case golimumab) from the serum. In case of the latter, TNF can be directly coated, or captured by a monoclonal antibody against TNF that was first coated onto the plate. The theoretical advantage of the latter is that TNF is always oriented in the same way. As the detecting antibody, either antihuman IgG, monospecific polyclonal ADAb (from immunised goats or rabbits) or monoclonal ADAb (murine origin) can be used. The advantage of a monoclonal or monospecific polyclonal ADAb is the specificity towards the TNF antagonist, resulting in lower nonspecific binding and a lower risk of false positives i.e., overestimation of drug levels. To measure ADAb concentrations, as coating and detection antibody, typically the TNF antagonist itself is used. The drug then forms a bridge between the capture and detection antibody. The final stage in all ELISA systems is a detection step. Unless a radioactive or fluorescent tag was used, this involves the introduction of an enzyme substrate. The enzyme converts the substrate to a detectable product. The intensity of signal produced when the substrate is added will be directly proportional to the amount of antigen captured in the plate and bound by the detection reagents. Enzyme-conjugated antibodies (especially those involving horseradish peroxidase, HRP) offer the most flexibility in detection and documentation methods for ELISA because of the variety of substrates available for chromogenic, chemifluorescent, and chemiluminescent imaging. The generated data are typically graphed with optical density (OD) (or fluorescence) versus concentration to produce a sigmoidal curve. Known concentrations of antigen are used to produce a standard curve and then these data are used to measure the concentration of

unknown samples by comparison to the linear portion of the standard curve. However, the design of ELISA methods and lack of standardisation may lead to differences in results. Rigorous control of assay performance is therefore required, regardless of its use in a research setting, in clinical routine, or drug development and establishment of clinical thresholds to guide appropriate dosing.

### Commercially available golimumab ELISA assays.

#### *Measurement of serum golimumab concentrations.*

In addition to several research immunoassays, there are also multiple commercially available kits for the measurement of golimumab serum levels and ADAb. Golimumab measurement is by either anti-IgG detection antibody or an antibody directed against golimumab. Manufacturers of CE marked kits include Theradiag, Immundiagnostik RIDASCREEN, Promonitor, and Sanquin Diagnostic services. Other assays have been developed, primarily for research purposes by KU Leuven and Janssen R&D. Variations in their performance can be attributed to variations in assay methodology between the kits. The assay developed by Sanquin is an in-house ELISA and uses TNF-alpha for capture and rabbit biotinylated anti-golimumab for detection (Martineau et al., 2017). The KU Leuven assay measures serum golimumab concentrations using a sandwich ELISA. Golimumab is captured between an immobilised monoclonal antibody (MA-GOM-1) and HRP-labelled MA-GOM-2 that targets a different epitope on the golimumab molecule (Danese et al., 2014; Sheridan et al., 2018). The Janssen R&D assay determines golimumab concentrations using an in-house developed ECLIA. Standard curve calibrators, quality control samples, and test samples are added together with biotinylated capture antibody and ruthenium-labelled detection antibody (directed against the idiotype of golimumab) to the appropriate wells of

streptavidin-coated MSD plates. The plates are then washed, the reading buffer is added, and the plates are read on an MSD sector image reader. In the KU Leuven, Sanquin, and Janssen assays, the anti-golimumab (‘catcher’) antibody is directed against the idiotype of golimumab (Adedokun et al., 2019). However, this is not the case for the LISA TRACKER assay. Golimumab concentration assay characteristics are summarised in Table 7.

**Table 7**
*Golimumab concentration assay characteristics (TMB, 3,3',5,5'-tetramethylbenzidine)*

| Manufacturer | Technique | Microplate/Particle Coating | Primary Conjugate | Secondary Conjugate | Detection |
|---|---|---|---|---|---|
| **Theradiag** | ELISA | TNF-alpha | Biotinylated antihuman IgG1 antibody | Streptavidin-HRP conjugate | TMB |
| | CLIA | TNF-alpha coated magnetic beads | anti-golimumab polyclonal antibodies conjugated to acridinium ester | N/A | Triggers (e.g., alkaline hydrogen peroxide) |
| **Sanquin** | ELISA | TNF-alpha | Rabbit biotinylated anti-golimumab antibody | N/A | TMB |
| **KU Leuven** | ELISA | Immobilised monoclonal antibody (MA-GOM-1) | HRP-labelled MA-GOM-2 | N/A | TMB |
| **Janssen R&D** | ECLIA | TNF-alpha | Biotinylated capture antibody | N/A | Ruthenium-labelled |

| Manufacturer | Technique | Microplate/Particle Coating | Primary Conjugate | Secondary Conjugate | Detection |
|---|---|---|---|---|---|
| | | | | | detection antibody |
| **Immundiagnostik** | ELISA | monoclonal anti-golimumab antibody | HRP-labelled anti-golimumab antibody | N/A | TMB |
| **RIDASCREEN** | ELISA | TNF-alpha | HRP-conjugated monoclonal antibody (MA-GOM171D8) | N/A | TMB |
| **Promonitor** | ELISA | anti-TNF-alpha monoclonal antibody bound to human TNF-alpha | HRP-conjugated anti-golimumab human monoclonal antibody | N/A | TMB |

Published data exist comparing the operating characteristics of commercially available kits (LISA TRACKER, Theradiag) with three research immunoassays (Sanquin, KU Leuven, and Janssen R&D). As part of a study involving 78 patients with UC, serum samples were analysed using all four ELISAs and results compared (Adedokun et al., 2019). Median serum golimumab levels were 4.5, 3.5, 4.9, and 2.4 ug/mL with Theradiag, Sanquin, KU Leuven, and Janssen R&D assay, respectively. The Pearson correlation coefficients between the assays were excellent (ranging from 0.90 to 0.97) and Bland-Altman analysis also demonstrated good agreement (Adedokun et al., 2019). As part of a detailed quartile analysis, agreement in the second quartile (within which the putative clinical decision point for induction lies at approximately 2.5ug/mL (Boland et al., 2019; Danese et al., 2014) was considered 'satisfactory' but noted to be lower between LISA TRACKER and the other assays.

The specificity of the three research assays for golimumab was confirmed by using the sera of patients treated with infliximab or adalimumab (10 of each). None of these samples yielded any positive results (100% specificity for golimumab) (Adedokun et al., 2019). The Theradiag LISA TRACKER kit was not included in this assessment because the technical sheet already specifies that sera of patients treated with anti-TNF-alpha containing a crystallisable (Fc) fragment such as infliximab, adalimumab, and etanercept may cross-react with this golimumab test, due to the use of an anti-IgG detection antibody.

i-Tracker is a novel CLIA. When used in conjunction with a random-access analyser, it can produce up to 60 measurements in an hour with initial results within 35 minutes. It also allows the possibility for samples to be analysed individually, rather than as a batch, as is the case for an ELISA. The method involves TNF-alpha coupled magnetic beads in suspension to which serum is added. Following a wash step ester acridinium conjugate is

added, which in the presence of specific triggers, results in the emission of light that can be quantified by a chemiluminescent analyser. CLIA also has extended measurement range compared with ELISA assays. See Figure 10 for an illustration of the i-Tracker chemiluminescent golimumab concentration assay method.

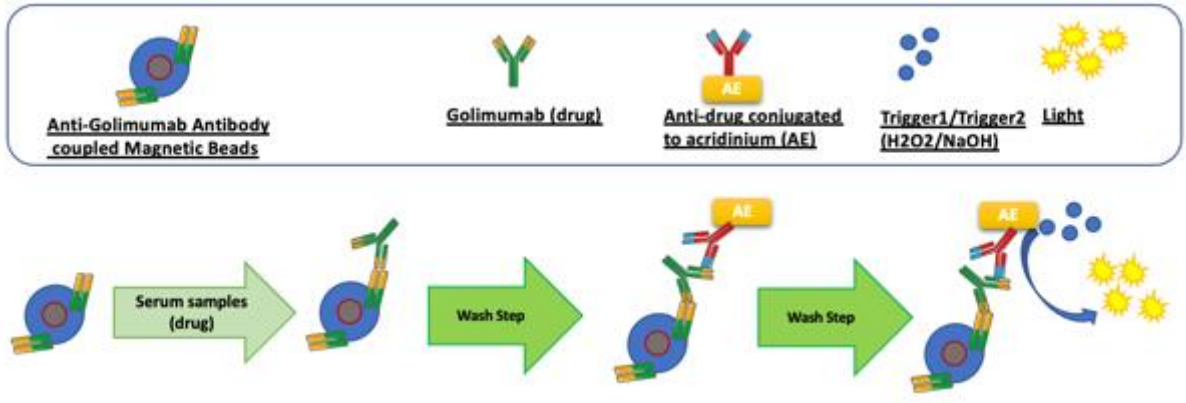



*Figure 10.* i-Tracker chemiluminescent golimumab concentration assay method

***Measurement of anti-golimumab antibodies.***

Three different assay methodologies for measuring anti-golimumab antibodies (AGA) have been evaluated alongside one another. Two of these use ELISA techniques, one drug-sensitive the other drug-tolerant, and the third was an RIA.

The Theradiag LISA TRACKER as well as the original assays from Janssen R&D and KU Leuven are all classical, first-generation, bridging, drug-sensitive ELISAs. For this type of assay, golimumab is coated onto a polystyrene microtiter plate. The plates are washed, and then blocked with 1% bovine serum albumin and the diluted serum sample is added to the antibody-coated well and allowed to bind. After washing, biotinylated golimumab is added and labelling is performed. This is using HRP-streptavidin, 3,3’,5,5’-tetramethylbenzidine (TMB) for Theradiag and Janssen R&D assays, and o-phenylenediamine for the KU Leuven assay. OD is then read using a spectrophotometer.

Subsequent to their original assay, Janssen R&D went on to design drug-tolerant ADAb ELISA, which allows for the measurement of antibodies in the presence of golimumab up to 12.5 ug/mL. This technique involves microplates precoated with streptavidin, which are then washed and then blocked with 1% bovine serum albumin in phosphate-buffered saline. A key step in achieving drug tolerance is incubation of the samples with acetic acid to dissociate immune complexes of antibody. The biotin-golimumab is then captured on the streptavidin-coated plate and the digoxigenin-golimumab is captured on the plate through a molecular bridge. Antidigoxigenin–HRP and TMB substrate are added, the reaction is stopped by adding sulphuric acid and absorbance is measured using a spectrophotometer.

The RIA developed by Sanquin involves incubation of samples with sepharose-immobilised protein A followed by washing with phosphate-buffered saline. Antidrug antibody binding is determined by overnight incubation with iodine 125–labelled F(ab)2 golimumab diluted in freeze buffer. Unbound label is then removed by washing and protein A–bound radioactivity is then measured (Philip et al., 2019; Sazonovs et al., 2018; Sheridan et al., 2018). Although drug-tolerant RIAs have been developed (van Schie et al., 2015), this particular assay is drug-sensitive.

ADAb assays use a monoclonal ADAb as a calibrator and antibody titres are thus expressed in relative units (e.g., ng/mL equivalents of the calibrator antibody) and absolute values cannot be directly compared between different assays. Therefore, rather than measure the quantity of antibody present, the frequency of samples with detectable antibodies was measured and compared. This demonstrated that the drug-sensitive assays (Theradiag, Sanquin, and KU Leuven), which can only detect ADAb in the setting of low or absent drug levels, identified antibodies in 2/78 (2.6%) of samples. For these patients, golimumab

quantification was under the limit of detection with the Sanquin and KU Leuven assays but was detectable with the Theradiag assay (concentration not detailed) (Adedokun et al., 2019). Using the drug-tolerant, modified Janssen ELISA, antibodies were detected in 22/78 (27.8%) of patients. However, as discussed previously, in other studies that have correlated the detection of additional antibodies with drug-tolerant assays with clinical outcomes, these may be of limited clinical relevance (Boland et al., 2019; Feuerstein et al., 2017).

Theradiag's novel anti-golimumab CLIA (i-Tracker) is a two-step immunoassay using microparticles and acridinium ester labelled chemiluminescent technology. In the first step, the golimumab coupled magnetic microparticles and serum sample are mixed in an assay cuvette, which allows AGA to bind to the surface of the microparticles. After incubation, unbound reagent and sample matrix are removed by washing and the microparticles-golimumab-AGA immunocomplex is kept with the help of a magnetic separator. Following this, golimumab conjugated to acridinium ester is added. After incubation, excess acridinium ester conjugate is removed by washing, and finally the light induced by acridinium ester is detected by addition of triggers. The relative light unit (RLU) intensity is proportional to the amount of AGA. According to a certain specific AGA RLU-concentration standard curve, the RLU obtained can be interpreted to anti-golimumab antibody concentration in the sample expressed as ng/mL. See Figure 11 for an illustration of the i-Tracker chemiluminescent anti-golimumab antibody concentration assay method. A summary of anti-golimumab antibody concentration assay characteristics is presented in Table 8.

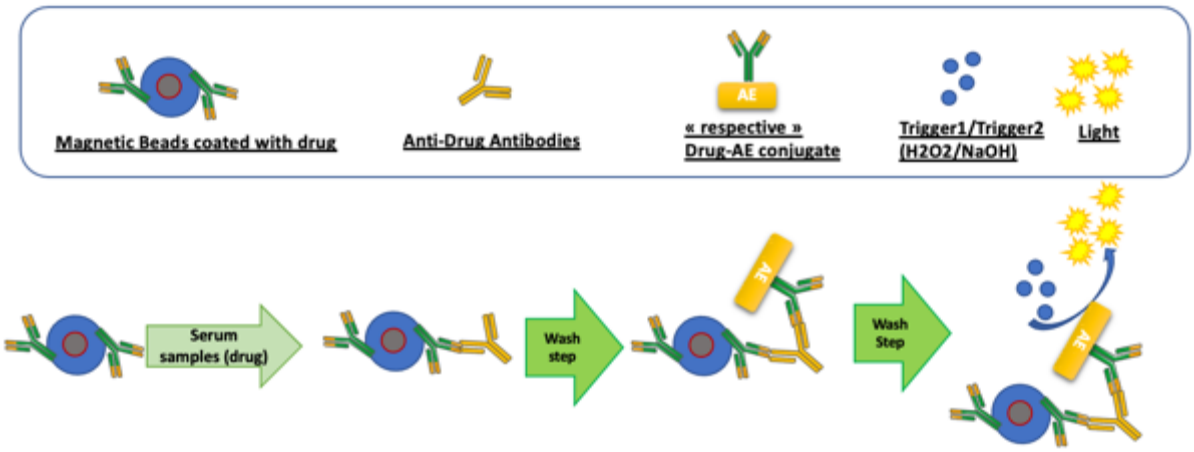



*Figure 11.* i-Tracker chemiluminescent anti-golimumab antibody concentration assay *method*

**Table 8**

*Summary of anti-golimumab antibody concentration assay characteristics*

| Manufacturer | Technique | Drug sensitivity | Microplate/particle coating | Primary conjugate | Secondary conjugate | Detection |
|---|---|---|---|---|---|---|
| **Theradiag** | ELISA | Sensitive | Golimumab | Biotinylated-golimumab | HRP-streptavidin | TMB |
| | CLIA | Sensitive | Golimumab-coated magnetic beads | Golimumab-conjugated acridinium ester | N/A | Triggers (e.g., alkaline hydrogen peroxide) |
| **Sanquin** | RIA | Sensitive | Sepharose-immobilised protein A | iodine 125–labelled F(ab)2 golimumab | N/A | protein A–bound radioactivity |
| **KU Leuven** | ELISA | Sensitive | Golimumab | Biotinylated-golimumab | o-phenylenediamine | TMB |
| **Janssen R&D** | ELISA | Both sensitive and tolerant versions available | Golimumab | Biotinylated-golimumab | HRP-streptavidin | TMB |
| **Immundiagnostik** | ELISA | Sensitive | Golimumab F(ab)2 fragments | HRP-conjugated golimumab | Biotinylated-infliximab | TMB |

| Manufacturer | Technique | Drug sensitivity | Microplate/particle coating | Primary conjugate | Secondary conjugate | Detection |
|---|---|---|---|---|---|---|
| **RIDASCREEN** | ELISA | Sensitive | Immobilised monoclonal antibody | biotin-conjugated golimumab | peroxidase-conjugated streptavidin | TMB |
| **Promonitor** | ELISA | Sensitive | Golimumab | HRP-conjugated golimumab | N/A | TMB |

***Collection of samples for golimumab TDM.***

TDM of biologic agents is traditionally based on trough concentrations acquired by venepuncture and requiring sample collection just before the next administration of the drug. Although this provides standardisation and has logistical advantages for intravenously administered drugs, it also has several disadvantages. For example, there is often a delay of several weeks or months between TDM being carried out, results being available to a clinician for interpretation, and finally for any necessary dose adjustments to be implemented. In addition, it may not be convenient, nor necessary, for subcutaneously self-administered drugs to have TDM performed at trough. Moreover, to adequately study absorption, distribution, and clearance of the drug, rich sampling is required (i.e., at intermediate time points as well as at trough). Evidence in this regard is currently limited but emerging and may, in the future, support the role of TDM, based on intermediate or peak concentrations (Rispens et al., 2012; van Schouwenburg et al., 2010).

To evaluate the use of dried blood spot (DBS) sampling for golimumab TDM, the KU Leuven group carried out the GOlimUmab Dried blood spot Analysis (GOUDA) study (Magro et al., 2019b). DBS sampling involves a finger prick (similar to glycaemia measurement) to apply whole blood to sampling paper. The sample papers can then be stored and transported at ambient temperature, opening up the possibility of remote monitoring by patients posting their samples directly to the laboratory. This type of self-sampling method has the potential to revolutionise TDM in clinical practice and also to facilitate rich sampling in a study setting, such as GOUDA. The initial phase of the study involved developing methodology to process DBS samples using blood from healthy donors, spiked with known concentrations of golimumab. This included evaluation of several different elution buffers and incubation

periods to optimise extraction recovery before measuring samples using an in-house ELISA. This yielded a mean of 54.4% (SD +/- 9.0%) and resulting correction factor for extraction of spiked golimumab in whole citrated blood of 1.8 (Magro et al., 2019b). The clinical part of the study included five UC patients who were commencing golimumab induction therapy and five receiving maintenance. DBS and concurrent serum samples were available at a total of 79 time points, demonstrating an overall excellent correlation, with a conversion factor of 3.9 between the two modalities (Magro et al., 2019b). An excellent correlation between the in-house assay and a commercially available ELISA (apDia/RIDASCREEN) was also observed for serum samples, although there was a systematic difference of 16%. There was, however, no correlation between golimumab trough concentrations and total exposure (using AUC analysis). Multiple peaks were observed in drug absorption but patients who achieved MH appeared to have fewer fluctuations in golimumab concentration (either judged by TL or AUC analysis) (Magro et al., 2019b). This type of nonlinear pattern of disposition has been frequently observed with biotherapeutics undergoing target-mediated drug disposition before reaching the systemic circulation (Verstockt et al., 2019). The mechanism responsible for this phenomenon is currently unknown but the authors postulate that first-pass catabolism of the drug at the subcutaneous administration site, or in the draining lymphatics, may be possible explanations.

## Assay Validation and Verification

The International Organisation for Standarization (ISO) document, *'Medical laboratories — Requirements for quality and competence'* (ISO15189) specifies the quality management system requirements particular to medical laboratories. This document defines both validation and verification in relation to assay evaluation. Validation is defined as

confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled. Verification is defined as confirmation, through provision of objective evidence, that 'specified' requirements have been fulfilled. ISO15189 states that validation of examination procedures is required for:

1. Non-standard methods
2. Methods designed or developed by the laboratory
3. Standard methods which are used outside their intended scope
4. Validated methods which are subsequently modified

A non-standard method may be considered as one that is not traceable to a reference (source) method. A laboratory designed or developed method may be 'standard' but in practice tends to be 'non-standard' by this definition.

Validation can be carried out in full, or partially, and although the definitions of these terms are not universally harmonised, there exists some consensus. This is generally required for *de novo* methods. Partial validation is required for those tests used outside their intended scope or following modifications of validated analytical methods. Although it has been suggested by some groups that partial validation should include all parameters except for robustness (on the basis that robustness should have been covered by the manufacturer during development), the International Organization for Standardization simply states that 'the validations shall be as extensive as are necessary to meet the needs in the given application or field of application.'

Assay verification is the usual procedure for methods which are available commercially, and which have undergone validation by the manufacturer, and has CE marking. The performance specifications from such validation studies are normally stated in

accompanying product literature. In accordance with ISO15189 (an international standard for quality and competence for use in medical laboratories), validated examination procedures require 'independent verification by the laboratory before being introduced into routine use.' However, if the method has been modified for use by the laboratory, evaluations beyond those of simple verification should be taken. Method verification should include an evaluation of imprecision and inaccuracy as a minimum. The initial goal of verification is to ensure that the performance claims for the method have been met in its intended use conditions.

For either method validation or verification, the information obtained regarding the performance of a method and its related properties are ultimately used to confirm whether or not the method is suitable for its intended use or application.

## Aims

We aimed to evaluate the CE marked LISA TRACKER golimumab ELISA kit on the automated DS2 platform and verify the following parameters:

1. Accuracy
2. Recovery
3. Reproducibility
4. Linearity
5. Method comparison - ELISA versus CLIA
6. Measurement of uncertainty
7. Traceability
8. Interference

9. Sample stability

## Materials and methods.

### Equipment.

The following equipment was used:

- The DYNEX DS2® system (see Figure 12): Fully automated 2-plate ELISA processing system for sample distribution, incubation, reagent addition, washing, and detection steps of assays.

*Figure 12.* DYNEX DS2® system

- i-Track10 analyser (see Figure 13): A random-access CLIA analyser.


*Figure 13.* i-Track10 analyser

**Reagents.**

The following reagents were used:

- LisaTracker Duo Golimumab (Theradiag): ELISA for quantitative determination of golimumab and anti-golimumab antibodies.
- i-Tracker (Theradiag): CLIA kits for quantitative determination of golimumab and anti-golimumab antibodies.

**Sample storage and preparation.**

Serum samples were collected in serum separator tubes (SST) and centrifuged at 3000 rpm for 10 minutes prior to storage at -20$^{o}$C. Frozen samples were thawed on a roller mixer and re-centrifuged prior to analysis. Thawed samples were stored at 2-8$^{o}$C for a maximum of 5 days prior to analysis. All samples were analysed within a year of collection.

**Method.**

All assays were performed using the LISA TRACKER (Theradiag, France), ELISA, and were automated on the DS2 automated ELISA machine. Assay parameters were programmed in accordance with manufacturer instructions incorporating all necessary validation criteria for assay acceptance including quality control.

**Golimumab drug and antidrug antibodies.**

Patient samples were pre-diluted (1 in 100 golimumab and 1 in 2 anti-golimumab). Calibrators and control were added to microwell plates coated with TNFα and golimumab-coated wells respectively. The steps for the tests are outlined in Table 9.

**Table 9**
*Reagents and procedure for LISA TRACKER ELISA (TDL, assay dilution buffer)*

| Reagents | Procedure |
|---|---|
| Standards<br>Diluted positive controls<br>Diluted samples | 100 uL / wells |
| Incubation | 1 hour at room temperature |
| Washing | Wash 3 times with TDL buffer: 3 x 300 uL / wells |
| Biotinylated antibodies | 100 uL / wells |
| Incubation | 1 hour at room temperature |
| Washing | Wash 3 times with TDL buffer: 3 x 300 uL / wells |
| HRP-Streptavidin | 100 uL / wells |
| Incubation | 30 minutes at room temperature |
| Washing | Wash 3 times with TDL buffer: 3 x 300 uL / wells |
| Substrate (TMB) | 100 uL / wells |

| Reagents | Procedure |
|---|---|
| Incubation | 15 minutes at room temperature. Protect from light. |
| Stop solution | 100 uL / wells |

**Specific reagents.**

The specific reagents used were:

- Biotinylated antihuman IgG1 antibody for golimumab.
- Biotinylated-golimumab for AGA.

**Calculation of results.**

- Golimumab: Calibration standard optical densities were automatically plotted using a four-parameter logistic (4-PL) curve fit from which patient results were extrapolated.
- Anti-golimumab antibodies: Calibration standard ODs were automatically plotted using a quadratic curve fit from which patient results were extrapolated.

# Results

**Accuracy.**

According to ISO 5725, accuracy can be defined as the closeness of agreement between independent test results obtained under stipulated conditions. Accuracy is difficult to quantify, and it is therefore, the inversely related imprecision that is commonly reported.

Accuracy was determined by generating spiked sample standards using samples provided by Theradiag with predetermined concentrations. See Table 10.

**Table 10**

*Summary of accuracy experiments (VIA, Viapath; GOL, golimumab; GOL1898 and GOL2978 refer to two different DYNEX DS2® automated analysis machines)*

| | | | GOL1898 | | GOL2978 | |
|---|---|---|---|---|---|---|
| | Mean | Target range | GOL | % difference from mean | GOL | % difference from mean |
| VIA GOL 31 | 2.2 | 1.3-3.1 | 2.2 | 0.0 | 1.9 | -13.6 |
| VIA GOL 32 | 3.8 | 2.2-5.4 | 3.8 | 0.0 | 3.5 | -7.9 |
| VIA GOL 33 | 4.8 | 3.8-5.8 | 4.6 | -4.2 | 4.0 | -16.7 |
| VIA GOL 34 | 1.6 | 1.3-1.9 | 1.5 | -6.3 | 1.4 | -12.5 |
| VIA GOL 35 | 0.7 | 0.6-0.8 | 0.8 | 14.2 | 0.8 | 14.2 |

**Recovery.**

The recovery of an analyte in an assay is the detector response obtained from an amount of the analyte added to and extracted from the biological matrix, compared to the detector response obtained for the true concentration of the analyte in the solvent. A spike recovery test was conducted to investigate if the concentration–response relationship is similar in the calibration curve and the samples. A bad outcome of the test suggests that there are differences between the sample matrix and calibrator diluent that affects the response in signal. Data obtained from this study could help to find a diluent mimicking the biological sample in which the calibrator and the native protein give the comparable detector signals all along the measuring range (Andreasson et al., 2015).

Recovery experiments were performed using a pre-filled golimumab pen. The 0.5 ml pen contained 50 mg of golimumab i.e., 100 mg/ml solution. Stocks were prepared using steroid-free serum (SFS) as a matrix as follows:

- 10 ug/mL SFS spiked stock prepared as follows:
    - 100 uL 100 mg/mL stock + 900 uL $dH_2O$ = 10 mg/mL
    - 100 uL 10 mg/mL stock + 900 uL $dH_2O$ = 1 mg/mL (1000 ug/mL)
    - 25 uL of this + 2475 uL SFS = 10 ug/mL SFS stock
- 5 ug/mL SFS spiked stock prepared as follows:
    - 50 uL 10 mg/mL stock + 950 uL $dH_2O$ = 500 ug/mL
    - 50 uL of 500 ug/mL + 4950 uL SFS = 5 ug/mL SFS stock.
- 2.5 ug/mL SFS spiked stock prepared as follows:
    - 25 uL of 500 ug/mL + 4975 uL SFS = 2.5 ug/mL SFS stock.

Recovery was tested using both SFS and assay dilution buffer (TDL). Samples were analysed using identical techniques. See Table 11, Table 12, Table 13, and Table 14.

**Table 11**
*Recovery in steroid-free serum (SFS)*

| SFS | Expected golimumab (ug/mL) | Measured golimumab (ug/mL) | Recovery (%) |
|---|---|---|---|
| **Neat GOL** | 5.00 | 6.11 | 122 |
| **GOL 1:2 SFS** | 3.06 | 3.25 | 106 |
| **GOL 1:5 SFS** | 1.22 | 1.41 | 115 |
| **GOL 1:10 SFS** | 0.61 | 0.70 | 115 |
| **GOL 1:25 SFS** | 0.24 | 0.18 | 74 |
| **GOL 1:50 SFS** | 0.12 | 0.10 | 82 |

**Table 12**
*Recovery in assay dilution buffer (TDL)*

| TDL | Expected golimumab (ug/mL) | Measured golimumab (ug/mL) | Recovery (%) |
|---|---|---|---|
| **Neat GOL** | 5.00 | 6.11 | 122 |
| **GOL 1:2 TDL** | 3.06 | 3.06 | 100 |
| **GOL 1:5 TDL** | 1.22 | 1.17 | 96 |
| **GOL 1:10 TDL** | 0.61 | 0.54 | 88 |
| **GOL 1:25 TDL** | 0.24 | 0.15 | 61 |
| **GOL 1:50 TDL** | 0.12 | 0.10 | 82 |

**Table 13**
*Recovery in SFS performed by two separate operators*

| SFS | Expected golimumab (ug/mL) | Operator 1 | | Operator 2 | |
|---|---|---|---|---|---|
| | | Measured golimumab (ug/mL) | Recovery (%) | Measured golimumab (ug/mL) | Recovery (%) |
| **10mg/ml** | 10.0 | 7.6 | 76 | 7.3 | 73 |
| **5mg/ml** | 5.0 | 3.6 | 72 | 3.9 | 78 |
| **2.5mg/ml** | 2.5 | 1.6 | 64 | 1.5 | 60 |

**Table 14**
*Recovery in TDL performed by two separate operators*

| | | Operator 1 | | Operator 2 | |
|---|---|---|---|---|---|
| TDL | Expected golimumab (ug/mL) | Measured golimumab (ug/mL) | Recovery (%) | Measured golimumab (ug/mL) | Recovery (%) |
| 10mg/ml | 10.0 | 7.0 | 70 | - | - |
| 5mg/ml | 5.0 | 3.4 | 68 | 3.2 | 64 |
| 2.5mg/ml | 2.5 | 1.7 | 68 | 1.5 | 60 |

**Reproducibility.**

Reproducibility is a measure of the variability of observed results whilst external factors (for example laboratory, technician, days, instrument and reagent lot) are kept constant and time between tests is minimal. Within-batch reproducibility was determined by analysing patient pools at two different concentrations. See Table 15.

**Table 15**
*Within-batch precision*

| | Pooled sample 1 | Pooled sample 2 |
|---|---|---|
| **Mean (ug/mL)** | 2.01 | 5.37 |
| **SD** | 0.16 | 0.37 |
| **Coefficient of variation (CV), %** | **7.8** | **6.98** |

**Linearity.**

Linearity describes the relative accuracy from recovery tests on the biological matrix or diluted matrix against the calibrators in a substitute matrix. The goal of investigating this is

to ascertain that the binding characteristic of the endogenous analyte to the antibodies is the same as for the calibrator (Andreasson et al., 2015). Linearity was evaluated in two diluents: SFS and TDL. Both showed good linearity. The slope for SFS was 1.1 with y-intercept 0.02 and x-intercept -0.02. Respective values for TDL were 1.0, -0.09 and 0.09 (see Figure 14).

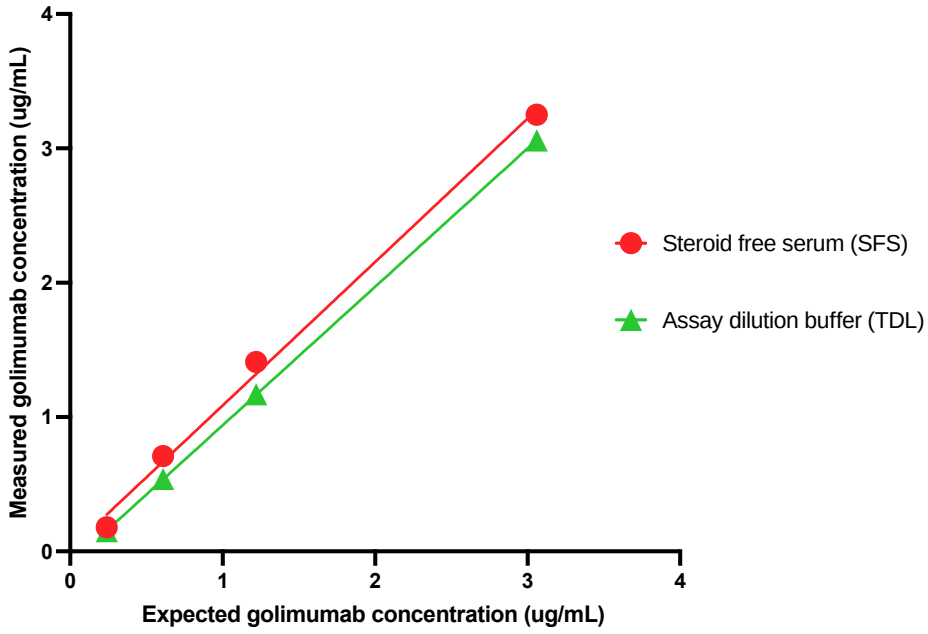



*Figure 14.* Linearity measured in SFS and TDL

**Method comparison.**

Method comparison was carried out by running 50 samples on both the LISA TRACKER ELISA as well as the i-TRACKER CLIA. Two samples (from a single patient) were marked outliers, so analyses were carried with, and without those data points.

Figure 15 and Table 16 present descriptive statistics for all data.

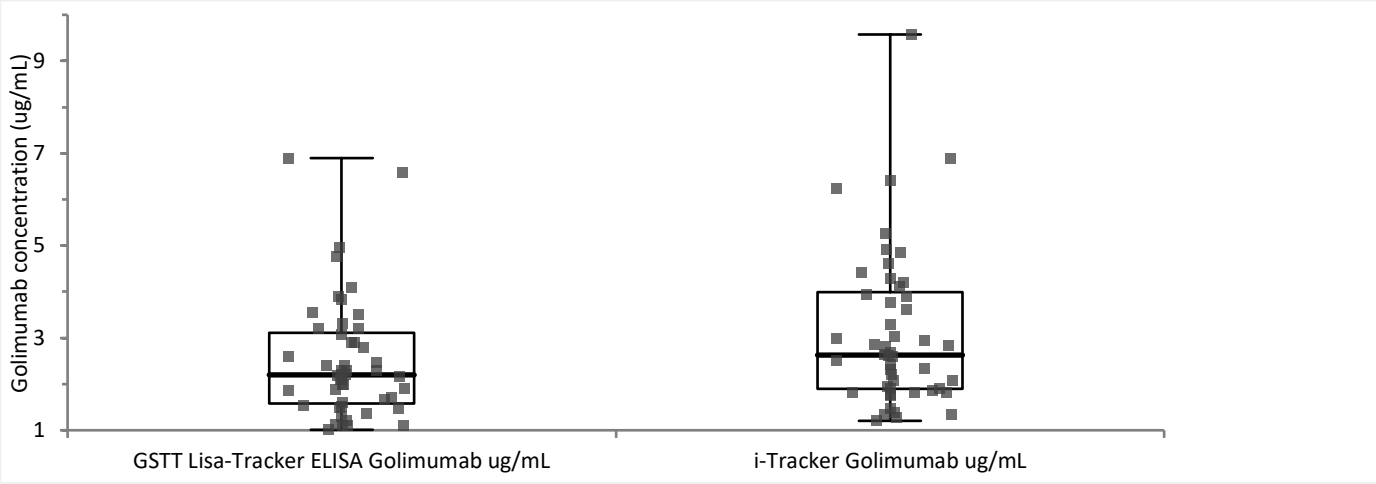



*Figure 15.* Method comparison – ELISA vs. CLIA (all samples)

**Table 16**

*Method comparison – ELISA vs. CLIA (all samples)*

| | Minimum | 1st Quartile | Median | 3rd Quartile | Maximum |
|---|---|---|---|---|---|
| **LISA TRACKER ELISA Golimumab ug/mL** | 1.01 | 1.58 | 2.20 | 3.11 | 6.90 |
| **i-Tracker CLIA Golimumab ug/mL** | 1.20 | 1.90 | 2.63 | 4.00 | 9.57 |

Figure 16 and Table 17 present descriptive statistics excluding the two outliers.

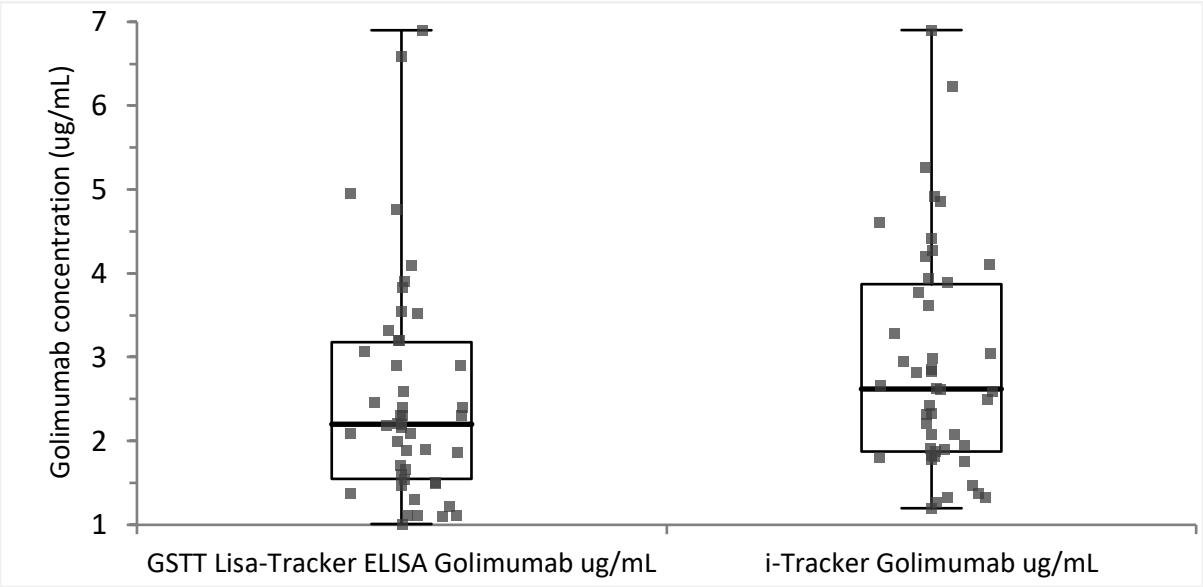



*Figure 16.* Method comparison – ELISA vs. CLIA (excluding two outliers)

**Table 17**

*Method comparison – ELISA vs. CLIA (excluding two outliers)*

| | Minimum | 1st Quartile | Median | 3rd Quartile | Maximum |
|---|---|---|---|---|---|
| **LISA TRACKER ELISA Golimumab ug/mL** | 1.01 | 1.55 | 2.20 | 3.18 | 6.90 |
| **i-Tracker CLIA Golimumab ug/mL** | 1.20 | 1.88 | 2.62 | 3.88 | 6.90 |

Figure 17 and Table 18 present the Passing-Bablok regression analysis for all data.

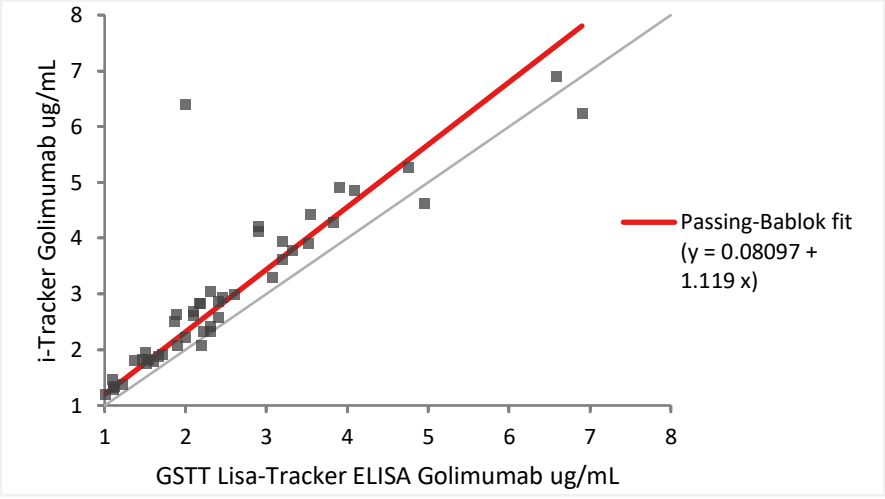


*Figure 17.* Passing-Bablok analysis comparing ELISA and CLIA (all samples)

**Table 18**

*Passing-Bablok analysis comparing ELISA and CLIA (all samples; GSTT, Guy's & St Thomas' Hospital)*

| **Passing-Bablok fit** | | | |
|---|---|---|---|
| **Equation** | i-Tracker Golimumab ug/mL<br>= 0.08097 + 1.119 GSTT Lisa Tracker ELISA Golimumab ug/mL | | |
| **Parameter** | **Estimate** | **Bootstrap 95% CI** | |
| **Intercept** | 0.08097 | -0.1275 | to 0.2923 |
| **Slope** | 1.119 | 1.027 | to 1.268 |
| CI based on 999 bootstrap samples. | | | |

Figure 18 and Table 19 present the Passing-Bablok regression analysis excluding the two outliers.

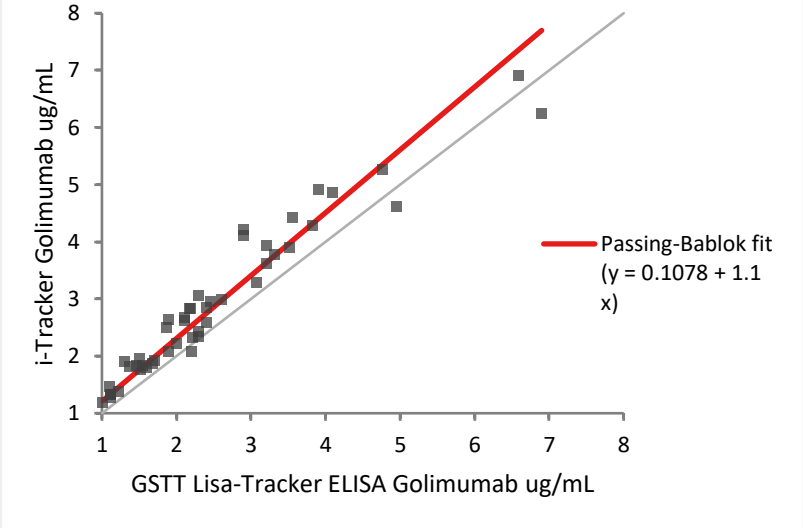



*Figure 18.* Passing-Bablok analysis comparing ELISA and CLIA (excluding two outliers)

**Table 19**

*Passing-Bablok analysis comparing ELISA and CLIA (excluding two outliers; GSTT, Guy's & St Thomas' Hospital)*

| **Passing-Bablok fit** | | | |
|---|---|---|---|
| **Equation** | i-Tracker Golimumab ug/mL<br>= 0.1078 + 1.1 GSTT Lisa Tracker ELISA Golimumab ug/mL | | |
| **Parameter** | **Estimate** | **Bootstrap 95% CI** | |
| **Intercept** | 0.1078 | -0.05825 | to 0.3350 |
| **Slope** | 1.100 | 1.019 | to 1.206 |
| CI based on 999 bootstrap samples. | | | |

Figure 19 and Table 20 present the Bland-Altman analysis for all data.

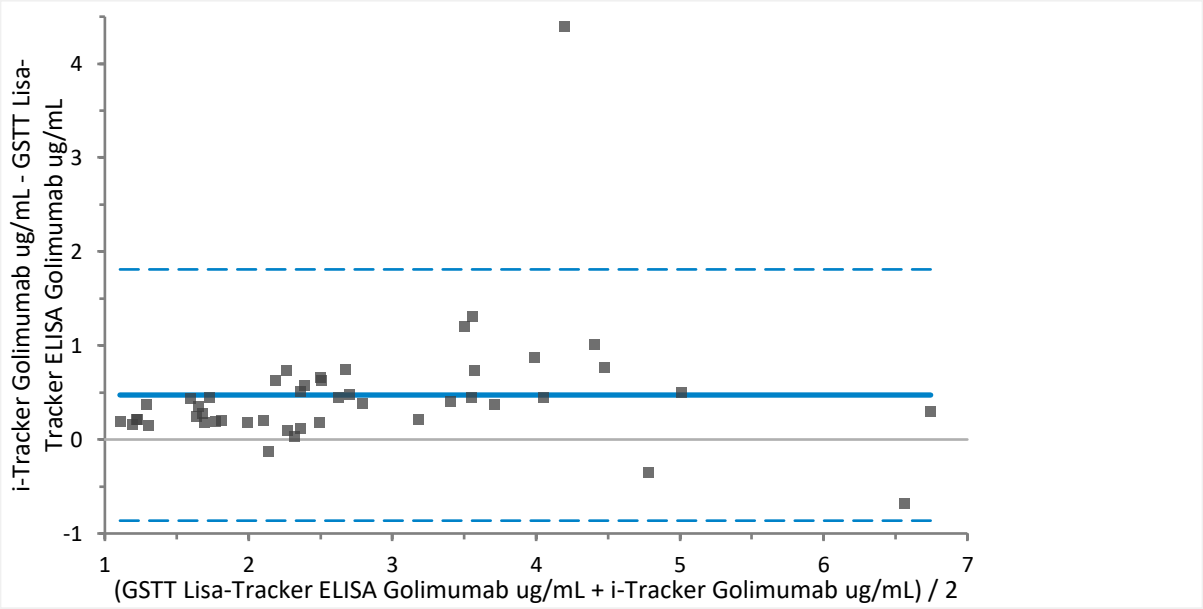



*Figure 19.* Bland-Altman analysis comparing ELISA and CLIA (all samples)

**Table 20**

*Bland-Altman analysis comparing ELISA and CLIA (all samples; LoA, limit of agreement)*

| **Parameter** | **Estimate** | **95% CI** | | **SE** |
|---|---|---|---|---|
| Mean difference | 0.473 | 0.2732 | to 0.6737 | 0.0995 |

| | | | | |
|---|---|---|---|---|
| 95% Lower LoA | -0.863 | -1.2081 | to -0.5187 | 0.1712 |
| 95% Upper LoA | 1.810 | 1.4656 | to 2.1550 | 0.1712 |
| SD | 0.682 | | | |

Figure 20 and Table 21 present the Bland-Altman analysis excluding the two outliers.

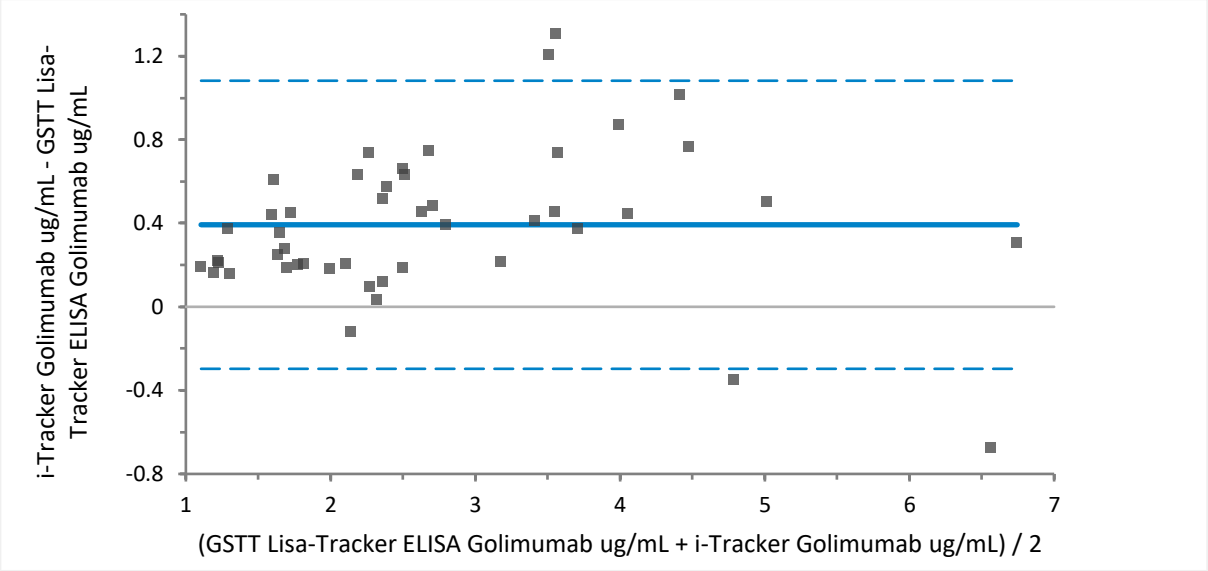



*Figure 20.* Bland-Altman analysis comparing ELISA and CLIA (excluding two outliers)

**Table 21**

*Bland-Altman analysis comparing ELISA and CLIA (excluding two outliers; LoA, limit of agreement)*

| Parameter | Estimate | 95% CI | | SE |
|---|---|---|---|---|
| **Mean difference** | 0.393 | 0.2895 | to 0.4961 | 0.0513 |
| **95% Lower LoA** | -0.297 | -0.4746 | to -0.1190 | 0.0883 |
| **95% Upper LoA** | 1.082 | 0.9045 | to 1.2602 | 0.0883 |
| **SD** | 0.352 | | | |

**Measurement of uncertainty.**

Anti-TNF drugs exhibit differing PD profiles based on route of administration / dose / frequency. Also, ADAb may only be present in some patients and will not be apparent until drug levels are sub-therapeutic. In this context, it is not possible to set targets for measurement of uncertainty based on biological variation. However, a range of control measures can be instituted to mitigate against measurement uncertainty. These can be divided into pre-analytical, analytical and post-analytical.

**Table 22**
*Pre-analytical control measures to limit measurement uncertainty*

| **Step** | **Measurement uncertainty** | **Control measure** |
|---|---|---|
| Test request | Incorrect requests may result in inappropriate analysis | GO-LEVEL specific request form designed to allow users to clearly indicate test required and study cohort and time point. Request forms checked for each sample before analysis and during validation. |
| Sampling | Correct time of sampling | As part of GO-LEVEL sample collection windows were protocolised such that during induction therapy (weeks 6, 10, and 14), all samples were collected within 4 days of the subsequent administration. During maintenance therapy all samples were collected within 7 days of the subsequent administration.<br>Upon review of sample timing, during induction samples were taken at median of 0 days (range 0-4 days) prior to the subsequent administration (i.e., on the same day). During maintenance, samples were taken at median of 2 days (0-6 days) prior to the subsequent administration. |
| Sample handling | Stability / sample integrity | The tests should be performed on serum or on plasma. Lipemic sera should be avoided, as well as samples which have been frozen and defrosted more than once. To avoid any nonspecific binding, samples which have been frozen for more than 6 months should be centrifuged and filtered. Samples should be visually inspected and cloudy samples should not |

| | |
|---|---|
| | be analysed. Samples should not be kept frozen (-20C) for over 3 years or undergo more than three freeze-thaw cycles before analysis. |

**Table 23**

*Analytical control measures to limit measurement uncertainty*

| Step | Measurement uncertainty | Control measure | Traceability |
|---|---|---|---|
| Sample dilution | Volumes of sample and diluent dispensed | Probe integrity maintained by maintenance procedures. Control material subjected to same dilution as patient samples. | Maintenance log and annual preventative maintenance records. Internal quality control recorded on Excel spreadsheet. |
| Calibration | Accuracy of results | Calibration curve generated with each kit. Validity of each curve is confirmed using manufacturer validation criteria and assessed by use of internal quality control. The top standard should have an OD of at least 0.8. | Lot number of calibrators recorded and traceable on validation sheet. |
| Reagent temperature | Reagent temperature has direct impact on ODs | Where sample storage is necessary, serum samples should be collected in SST and centrifuged at 3000 rpm for 10 minutes prior to storage at -20$^{o}$C. Prior to analysis, frozen samples should be thawed on a roller mixer and re-centrifuged. Once thawed, samples should be stored at 2-8$^{o}$C for a maximum of 5 days prior to analysis. | Competency records for staff and standard operating procedure |

| Step | Measurement uncertainty | Control measure | Traceability |
|---|---|---|---|
| Environment | Temperature has direct effect on optical densities obtained for standards and patient samples | Temperature monitoring of laboratory. Assays will not be attempted if room temperature outside of acceptable limits (18-25°C). | Temperature monitoring. Also recorded on each assay sheet. |
| Operator variability | Inconsistency in procedures performed | Standard operating procedure and competency in place and regularly reviewed. | Recorded on competency assessment. |

**Table 24**

*Post-analytical control measures to limit measurement uncertainty*

| Step | Measurement uncertainty | Control measure |
|---|---|---|
| Interpretation and authorisation | Subjectivity | Interpretation of drug concentration and ADAb results requires an appreciation of multiple factors, including but not limited to, golimumab treatment time point, current dosing, and UC disease state, co-therapy with immunomodulatory medication, prior anti-TNF exposure, response, and TDM results. |
| Quality of results | Accuracy and bias of results generated | Internal quality control procedures in place. Data used to monitor MU periodically. Sample exchanges in place with two centres, data from which is reviewed and summarised on an electronic quality management system (Q-Pulse). |

**Traceability.**

Theradiag's golimumab assays are calibrated against golimumab drug material from manufacturer Janssen Biotech, Inc. (formerly Centocor Biotech, Inc.) and provided by MSD in Europe. Their anti-golimumab antibody assays are calibrated against our own standard made from polyclonal AGA produced in rabbits.

Patient results for drug and ADAb are calculated automatically by the DS2 software based on extrapolation of absorbance/OD against the standard curve. For drug assays the equation of the curve is determined by a sigmoid log-lin curve whereas for ADAb assays, this is determined by a quadratic log-lin analysis.

**Interference.**

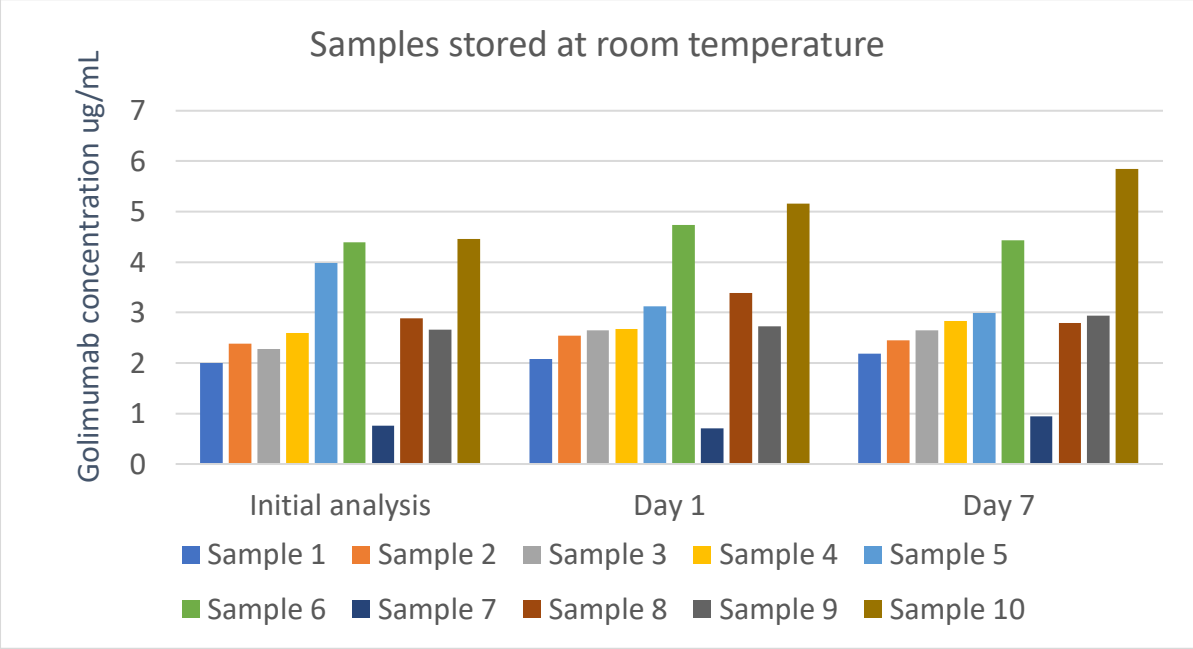
The LISA TRACKER kit insert states 'Serums of patients treated with anti-TNF containing a Fc fragment (infliximab, etanercept, adalimumab) may cross-reacted with golimumab test.' To confirm this, we analysed four serum samples taken from patients receiving other anti-TNF agents; one adalimumab treated patient, two on infliximab and one on etanercept. None of these patients were concurrently receiving golimumab. The results generated by analysing these samples using Theradiags LISATRACKER for golimumab demonstrated measurable concentrations of adalimumab (5.7 ug/ml), infliximab (2.9 ug/ml and 5.8 ug/ml) and etanercept (4.0 ug/ml).

**Sample stability.**

Sample stability can be defined as the chemical stability of an analyte in a given matrix under specific conditions for given time intervals. Sample handling prior to analysis has the potential to dramatically influence the results of a measurement. It is, therefore, important to investigate whether different storage conditions contribute to systematic errors in order to provide appropriate sample collection and transport instructions (Andreasson et al.,

2015). The results also have implications laboratory processes, such as how samples should be stored until analysis or pending a possible need for a re-run.

Samples were stored for at least 24 hours at room temperature, 4°C and at -20°C before analysis. Samples were re-analysed again a week later whilst stored at the same conditions i.e., room temperature, 4°C and at -20°C. To assess the impact of freeze/thaw cycle, a sample kept at -20°C was subjected to one freeze-thaw cycle and analysed in parallel with a sample that was kept at -20°C and was not subjected to a freeze/thaw cycle.



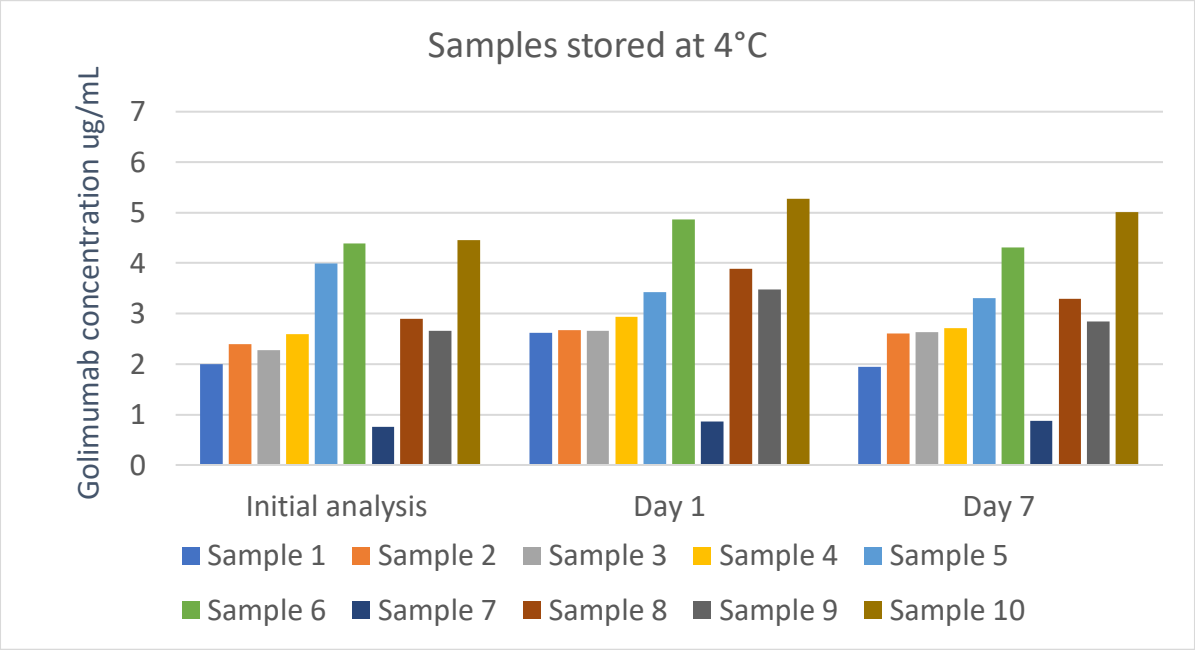



*Figure 21.* Sample stability at room temperature (above) and 4°C (below)

As analyses were run in batches, all samples underwent at least one freeze-thaw cycle. The impact of a second and third freeze-thaw cycle was evaluated. See Figure 22.

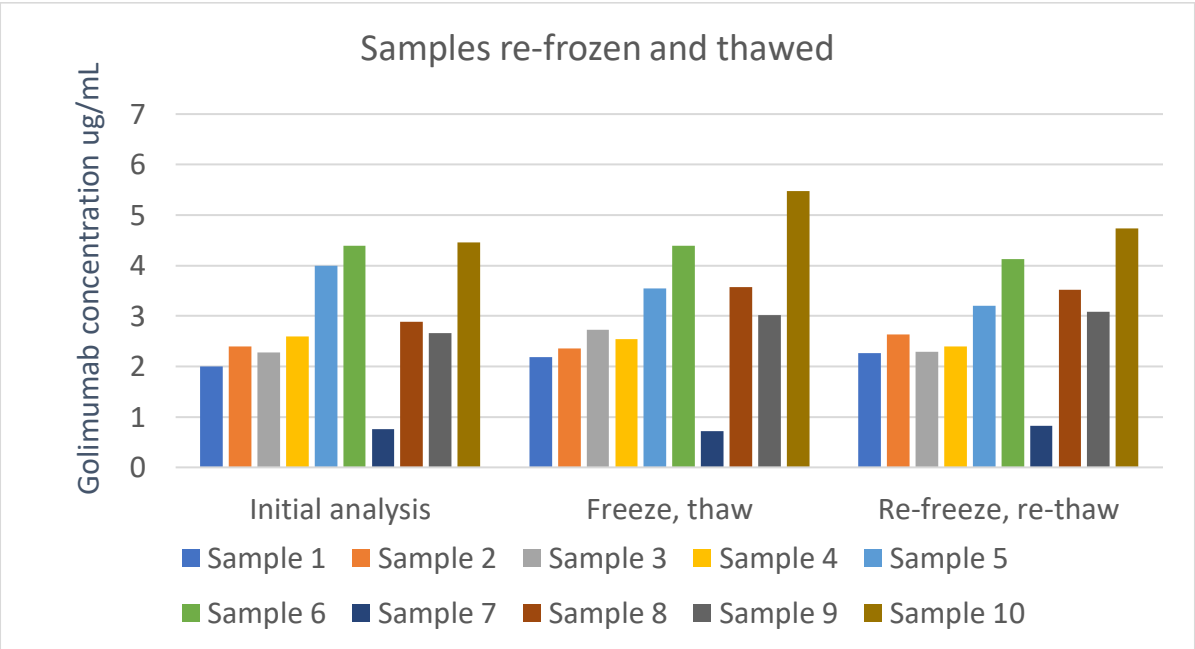



*Figure 22.* Sample stability – freeze-thaw

## Conclusions

Our assay verification exercise has demonstrated that the Theradiag LISA TRACKER has adequate operating characteristics for use in clinical practice. Our results with regards to accuracy, recovery, reproducibility, and linearity were broadly within acceptable limits. Accuracy ranged from -13.6% to 14.2% and rates of recovery from 61% to 122%. Reproducibility was good, reflected by a coefficient of variation (CV) of approximately 7%. For linearity, the slope was very close to 1, and the x-, and y-intercepts were close to 0.

We also demonstrated the relative stability of samples kept under different conditions and confirmed the known potential for interference of assay results by other anti-TNF agents.

The analyses presented above are in keeping with previous similar analyses performed by the manufacturer and would support continued use of LISA TRACKER for golimumab serum concentration measurement. However, we also took the opportunity to compare Theradiag's LISA TRACKER ELISA with their recently developed i-Tracker CLIA to generate novel assay verification data. Through the combination of descriptive statistics, Bland-Altman, and Passing-Bablok regression analyses, we were able to demonstrate adequate correlation between the methods. As i-Tracker offers the practical benefits of random-access analysis, such as the possibility for samples to be processed individually (rather than as a batch), it has the potential to become widely adopted for use in routine clinical practice.

# Chapter 3: Golimumab Observational Effectiveness Study

## Background

Before the advent of biologic therapies, options for UC treatment primarily consisted of the stepwise use of mesalazine, corticosteroids, and immunomodulators. Although a large proportion of patients respond to mesalazine treatment alone (Sutherland & MacDonald, 2006a; Sutherland & MacDonald, 2006b), a significant minority require additional agents. However, corticosteroids are inappropriate for long-term use and thiopurine intolerance is relatively common (Goel et al., 2015). In addition, despite optimisation, some patients have disease that remains refractory to immunomodulation (Louis, Irving & Beaugerie, 2014). There is now good evidence from large-scale, RCTs demonstrating the efficacy of anti-tumour necrosis factor (anti-TNF) agents in this sub-group of patients (Samaan et al., 2014a). Following a NICE multiple technology appraisal (TA329) in 2015, all three anti-TNF agents (infliximab, adalimumab and golimumab) were granted approval for use in moderate-to-severe UC. These approvals have led to significant changes in UC treatment paradigms in the UK with associated patient benefit (Samaan & Irving, 2016).

Golimumab is a transgenic, fully human monoclonal immunoglobulin G1 antibody that is synthesised from TNF-immunised transgenic mice expressing human immunoglobulin G (Hutas, 2008; Lonberg, 2005). It is administered subcutaneously and is licensed for the treatment of UC, rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. NICE approval for UC was granted based on the efficacy demonstrated during an integrated phase 2 and 3 trial programme (PURSUIT: Sandborn et al., 2014a; Sandborn et al., 2014b). During the induction phase (PURSUIT-SC), golimumab-treated patients achieved a response

(defined as a decrease in Mayo score by at least 3 points and by 30% or more, with a bleeding subscore of 0 or 1, or decrease ≥1) significantly more frequently than those on placebo, thereby meeting the induction primary endpoint. Two different induction regimens were investigated and after 6 weeks of treatment, just over 50% of golimumab-treated patients had responded, compared with 30% in the placebo group ($p<0.0001$) (Sandborn et al., 2014b). NICE approval allows treatment at 200 mg at week 0, 100 mg at week 2 and weight-based dosing at week 6 (100 mg for patients weighing 80 kg and over, or 50 mg for those under 80 kg).

During the maintenance phase of the clinical trials programme (PURSUIT-M), patients were randomised to either 50 or 100 mg every 4 weeks. The response observed during induction was demonstrated to be durable, with 47% and 50% of patients, respectively, achieving a sustained response, compared with 31% in the placebo group ($p=0.010$ and $p<0.001$, respectively) thus, meeting the maintenance primary endpoint (Sandborn et al., 2014a). On the basis of weight-based differences in response rates seen in PURSUIT-M, the EMA (and subsequently NICE) approved the use of 4-weekly maintenance therapy at a dose of 100 mg for patients weighing 80 kg and over, or 50 mg for those under 80 kg. It should be noted that this differs from the US FDA approval, which allows 100 mg, 4-weekly for all patients, regardless of weight. An additional point of interest regarding PURSUIT-M was the stringent definition of maintained response; long-term continuous efficacy was evaluated over the course of 15 prospective assessments over 54 weeks, without loss of response permitted at any time point. This compares with only three assessments undertaken as part of the ACT-1 (Rutgeerts et al., 2005) maintenance trial and two in the ACT-2 (Rutgeerts et al., 2005) and

ULTRA (Sandborn et al., 2012) maintenance trials (the landmark RCTs of infliximab and adalimumab in UC, respectively).

### Aims

Although the data generated by large-scale, RCTs represent high-quality evidence (Burns, Rohrich, & Chung, 2011), there is growing appreciation of the importance of observational, real-life data in IBD (Salleron et al., 2016). *Effectiveness* relates to how well a treatment works in clinical practice, which is different from *efficacy*, which relates to how well it works in clinical trials (NHS, 2019). Using the cohorts from Guy's & St Thomas' Trust Hospital (GSTT) and King's College Hospitals (KCH) we aimed to contribute to the growing body of existing observational data from Europe (Bosca-Watts et al., 2016; Castro-Laria et al., 2016; Detrez et al., 2016; Taxonera et al., 2016; Varvara et al., 2016) and the US (Bressler et al., 2016; Hamed et al., 2014), the majority of which was available only in abstract or letter form (Renna et al., 2016; Tursi et al., 2016a; Tursi et al., 2016b).

## Methods

We performed a retrospective cohort study by reviewing prospectively maintained clinical records for all patients commencing golimumab at GSTT or KCH between September 2014 and January 2017. We screened the records of 58 patients, who received at least one injection of golimumab for UC during this data collection period. A single patient who had not completed at least the 6-week induction regimen was excluded (they received only the first dose before their disease worsened and they underwent emergency colectomy). Records of the remaining 57 patients were reviewed. Demographic information as well as the following disease-related data were collected: disease duration, distribution and

behaviour using the Montreal classification (Satsangi et al., 2006), duration of golimumab treatment, prior anti-TNF exposure, concomitant immunomodulation and weight (see Table 25). We also collected data regarding how often we deemed it necessary to dose escalate during maintenance therapy from 50 mg to 100 mg, 4-weekly. Dose escalation is not an option for patients weighing 80 kg or more, who would have been on 100 mg as standard and in whom there is, therefore, no scope for dose escalation within our pathway.

Our primary outcome of interest was the clinical effectiveness of golimumab in reducing UC clinical disease activity. This was evaluated using the SCCAI, which ranges from 0-19 with higher scores indicating increasingly active disease. Where available, we compared paired evaluations, taken prior to treatment initiation and again at the first clinical review after completing the 6-week induction regimen. Rather than being fixed, these time points varied from patient to patient with post-induction clinical assessments (SCCAI) being carried out at a median of 12 weeks from treatment initiation. Post-induction CRP and FC measurements were made at medians of 8 and 10 weeks, respectively. Treatment outcomes were predefined as follows: clinical response was defined as a reduction of 3 or more in SCCAI, clinical remission was defined as a SCCAI less than 3. These definitions were based on their previously demonstrated (partial) validity (Higgins, 2005) and our own previously published post-marketing experience of vedolizumab, in an attempt to aid comparability of results (Samaan et al., 2016).

Secondary outcomes included the effect of golimumab on biochemical markers of disease activity, endoscopic outcomes, rates of corticosteroid use, and the need for colectomy. Where available, biochemical disease activity was assessed using paired pre-initiation and post-induction serum CRP and FC measurements. A CRP of 5 mg/L or greater, was

considered biochemical evidence of disease activity. A FC value of less than 150 µg/g was considered indicative of biochemical remission (Mosli et al., 2015). Endoscopic Mayo scores of 0 or 1 were considered to represent MH (Samaan et al., 2014b). Dose-response and dose-remission analyses were carried out using maintenance dose (either 50 mg or 100 mg) and body weight (kg) at treatment initiation, to calculate each patient's dose received on a mg/kg basis. Rates of corticosteroid usage at each time point were also collected and colectomy was included in our outcome assessments if it occurred whilst on golimumab therapy. Figure 23 presents the study design and evaluations.

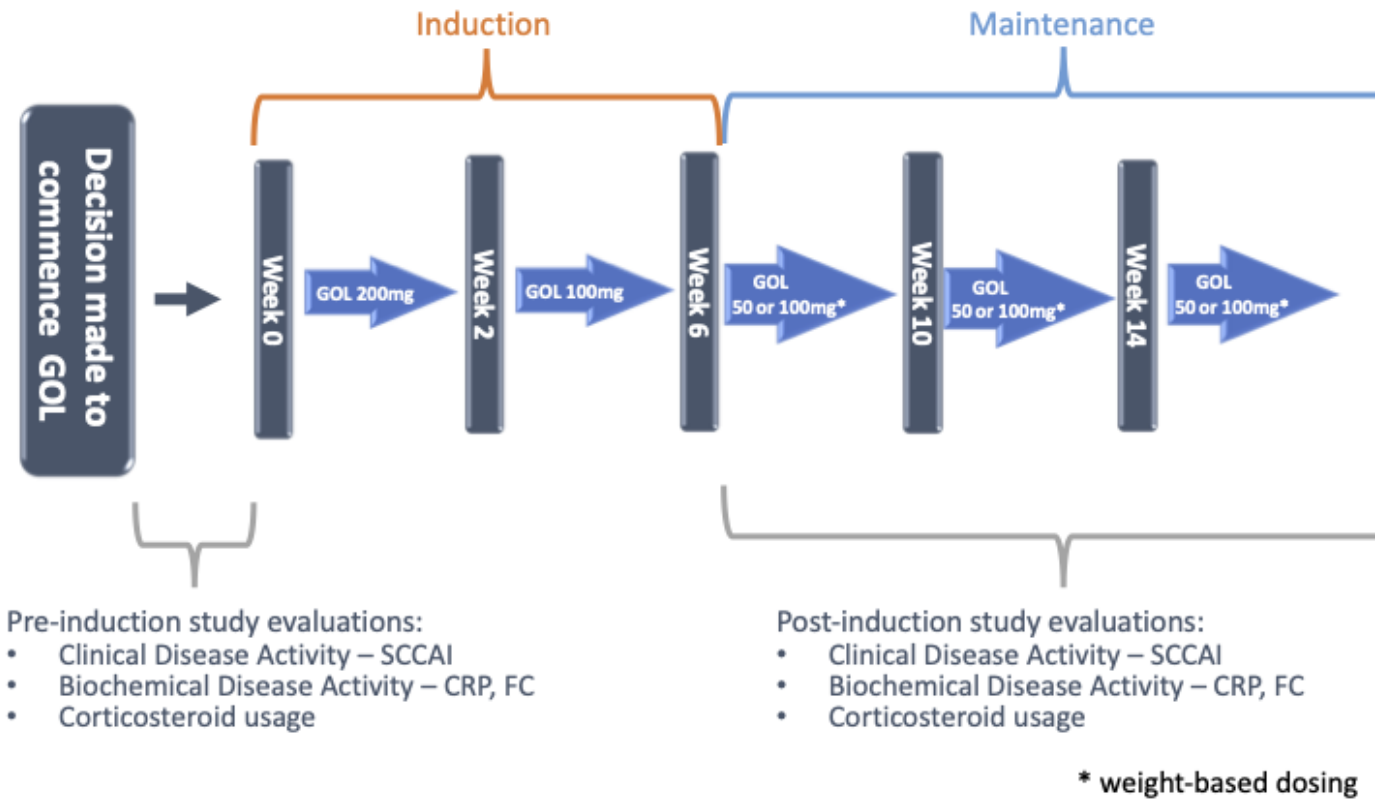



*Figure 23*. Study design and evaluations (GOL; golimumab, CRP; C-reactive protein, FC; faecal calprotectin, SCCAI; Simple Clinical Colitis Index)

## Statistical Analyses

Continuous data are summarised as medians and range (in brackets). Paired SCCAI, CRP, and FC values were compared using the Wilcoxon signed-rank test. Categorical variables were compared using the Fisher's exact test (GraphPad Prism v7.0a). Unless stated, p values are non-significant. All data below/above the limit of quantification were substituted with the value of the lower/upper limit of quantification, i.e., CRP 1 mg/L for levels of <1 mg/L, and FC 4800 µg/g for levels >4800 µg.

### Ethical Considerations.

The Health Research Authority does not consider post-marketing surveillance, research and therefore, suggests that NHS REC approval was not necessary. The study was registered with audit departments at both GSTT and KCH.

# Results

**Table 25**
*Baseline characteristics*

| Characteristic | n = 57 |
|---|---|
| **Gender, male: female** | 38:19 |
| **Median age at time of commencing golimumab (range), years** | 35 (20-72) |
| **Median disease duration (range), years** | 5 (1-52) |
| **Median duration of golimumab treatment (range), months** | 7 (2-28) |
| **Concomitant immunomodulator** | |
| ***Thiopurine*** | 41 (72%) |
| ***Methotrexate*** | 2 (4%) |

| | |
|---|---|
| ***None*** | 14 (24%) |
| **Previous anti-TNF experience** | |
| ***Naïve*** | 40 (70%) |
| ***Exposed*** | 17 (30%) |
| **Disease extent** | |
| ***Proctitis*** | 6 (11%) |
| ***Left-sided*** | 20 (35%) |
| ***Extensive*** | 31 (54%) |

**Clinical disease activity.**

Paired pre- and post-induction SCCAI values were available for 31 patients. Prior to commencing golimumab (pre-induction) the median SCCAI was 7 (range 2-19). The corresponding post-induction score had fallen significantly to 3 (0-11, $p<0.0001$). The median duration to assessment of this post-induction clinical disease activity score was 12 weeks (range 6-36 weeks) from initiation of treatment (see Figure 24).

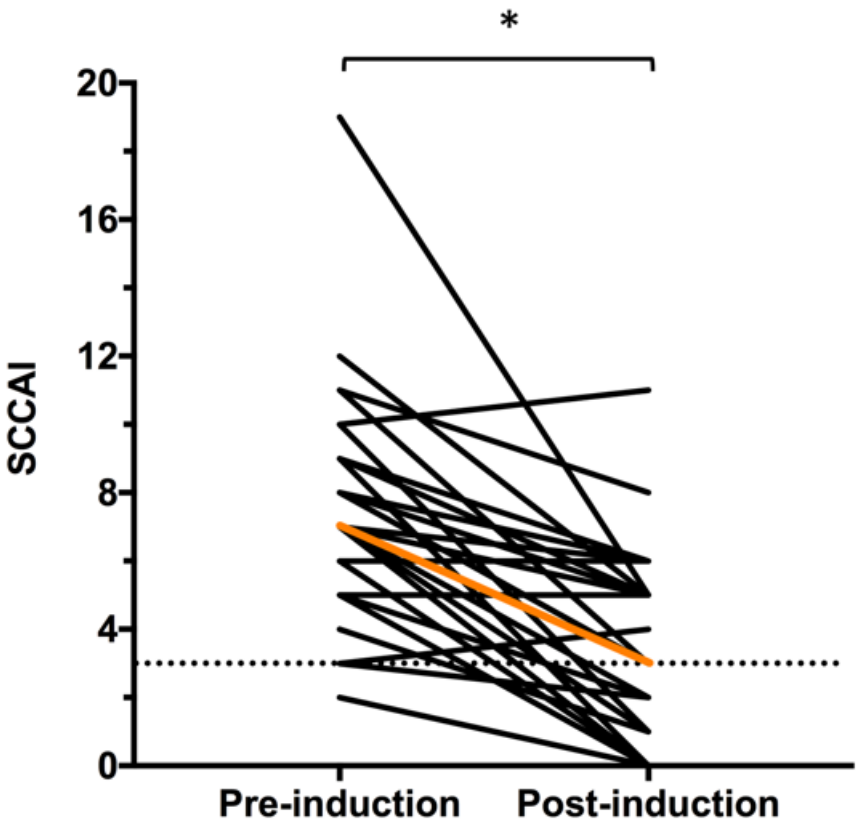



*Figure 24.* Change in SCCAI for patients with paired pre- and post-induction data available (n = 31, *p<0.0001). Post-induction scores assessed at a median of 12 weeks following treatment initiation.

**Response and remission at week 14.**

In addition to the 31 patients in our cohort with paired pre- and post-induction SCCAI data, there were a further 13 who discontinued treatment due to non-response, judged by their supervising physician but did not have documented, paired SCCAI scores. This group included patients who discontinued treatment at any time and for either primary or secondary non-response (loss of response). For the purposes of following response and remission rate analyses, these 13 patients were included in the non-response/non-remission groups, increasing the cohort to 44 (see Figure 25). Amongst this cohort 23/44 (52%) had a clinical response to golimumab and 15/44 (34%) achieved remission. The rate of corticosteroid-free remission was 13/44 (30%).

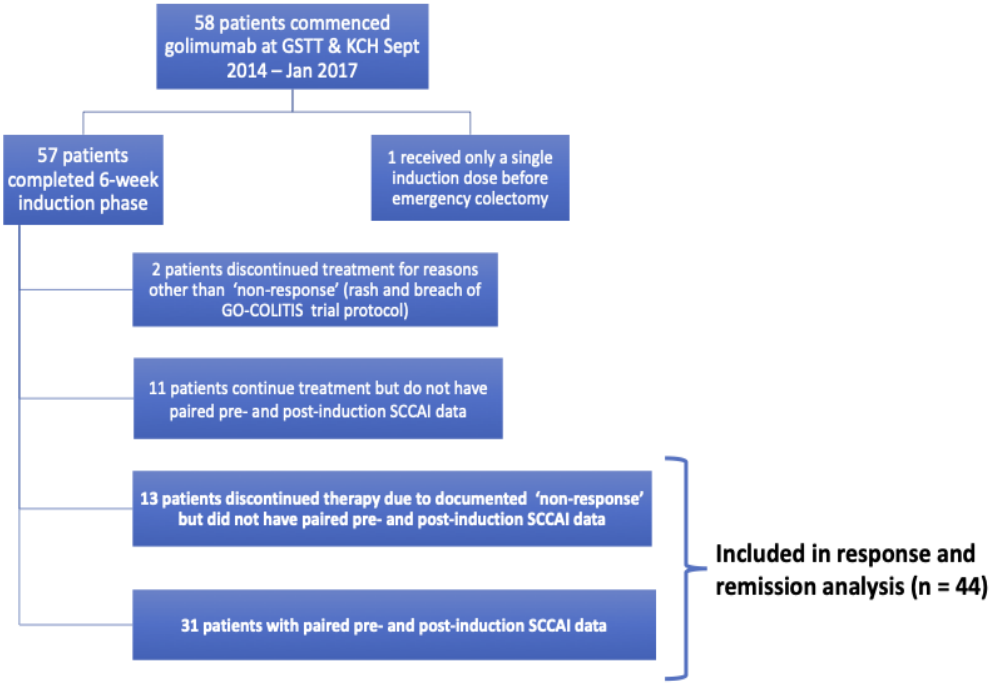



*Figure 25.* Patient inclusion algorithm

Rates of response, remission and corticosteroid-free remission (n = 44) are presented in Figure 26. Response was defined as SCCAI reduction ≥3. Remission was defined as SCCAI <3. Post-induction scores were assessed at a median of 12 weeks (range 6-36 weeks) following treatment initiation.

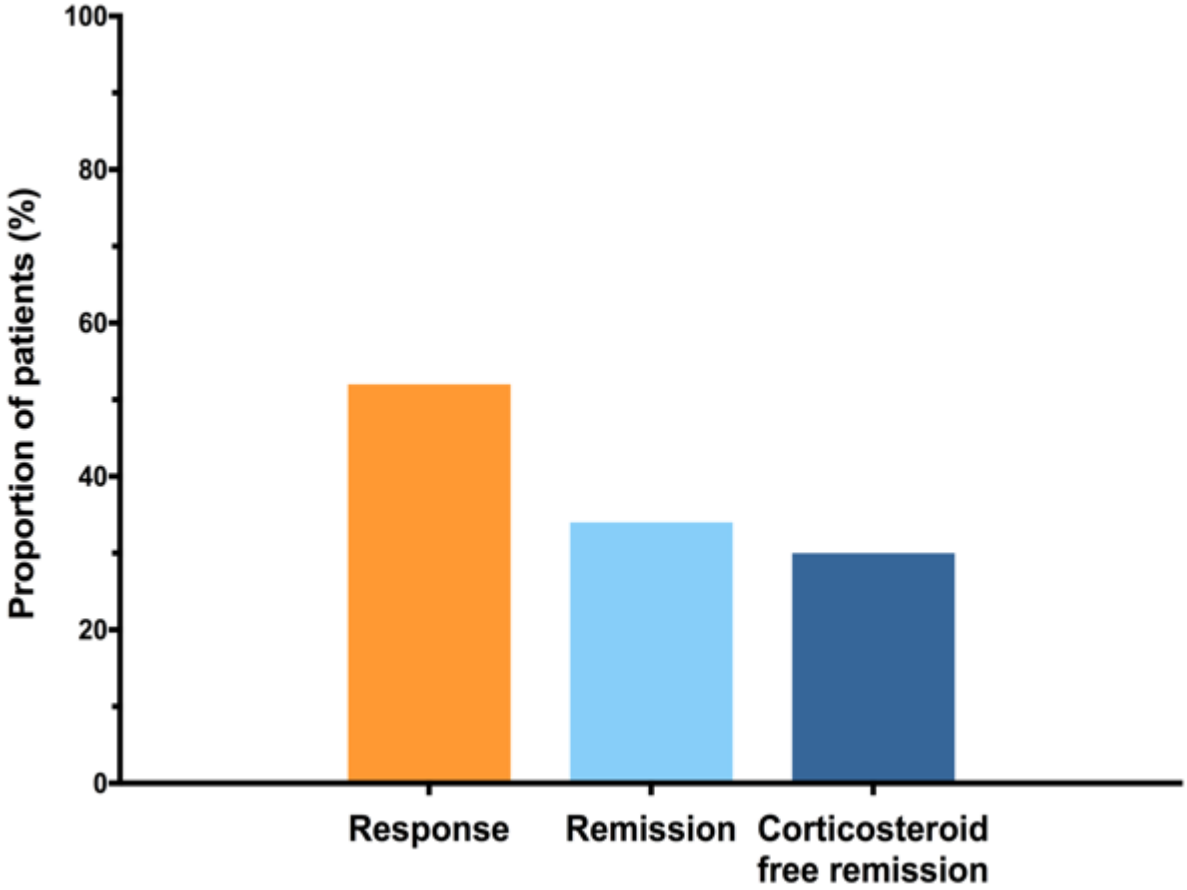



*Figure 26*. Rates of response, remission and corticosteroid-free remission (n = 44) at first post-induction assessment (median week 12).

Dividing patients by prior anti-TNF exposure, clinical response rates were significantly higher amongst anti-TNF naïve (20/31, 65%) than anti-TNF exposed patients (3/13, 23%: p=0.020). Corresponding remission rates were 13/31 (42%) and 2/13 (15%), respectively and the difference did not reach statistical significance (p=0.16). Dividing patients by those receiving golimumab monotherapy or in combination with an immunomodulator, the response rates were 5/13 (38%) and 18/31 (58%), respectively. The rates of remission were 5/13 (38%) for monotherapy and 10/31 (32%) for combotherapy. Other than prior anti-TNF exposure and maintenance dose on a mg/kg basis, no other predictors were evident in our univariate analysis (see Table 26).

**Table 26**
*Univariate analysis of predictors of response*

| | **Responders n (%) median (range)** | **Non-responders n (%) median (range)** | **p-value** |
|---|---|---|---|
| *n* | *23* | *21* | |
| Gender (Male vs. female) | 14 vs. 9 (61% vs. 39%) | 14 vs. 7 (66% vs. 33%) | 0.76 |
| Concomitant immunomodulator (Monotherapy vs. combotherapy) | 5 vs. 18 (22% vs. 78%) | 8 vs. 13 (38% vs. 62%) | 0.33 |
| Age, years | 34 (18-81) | 38 (20-62) | 0.60 |
| Duration of disease, years | 5 (0.3-18) | 5 (1.5-30) | 0.66 |
| **Prior anti-TNF experience (Exposed vs. naïve)** | **3 vs. 20 (13% vs. 87%)** | **10 vs. 11 (48% vs. 52%)** | **0.020*** |
| Maintenance dose (50mg vs. 100mg) | 13 vs. 10 (57% vs. 43%) | 12 vs. 9 (57% vs. 43%) | >0.99 |
| **Maintenance dose (mg/kg)** | **0.94 (0.63-1.92)** | **0.79 (0.64-1.17)** | **0.046*** |
| Corticosteroids at initiation (Corticosteroids vs. none) | 12 vs. 11 (52% vs. 48%) | 8 vs. 13 (38% vs. 62%) | 0.38 |

Rates of response, remission and corticosteroid-free remission when dividing patients by prior anti-TNF exposure (n = 44, *p=0.020) are presented in Figure 27. Response was defined as SCCAI reduction ≥3. Remission was defined as SCCAI <3. Post-induction scores were assessed at a median of 12 weeks (range 6-36 weeks) following treatment initiation.

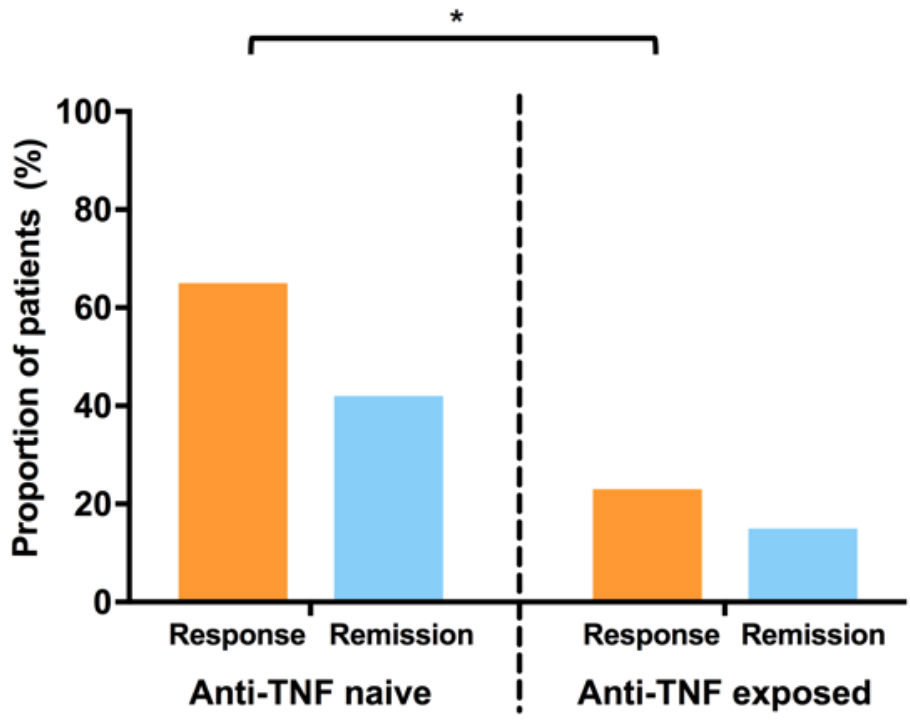



*Figure 27.* Rates of response, remission and corticosteroid-free remission when dividing patients by prior anti-TNF exposure

***Weight-based dose-response analysis***

When each patient's individual maintenance dose was calculated on a mg/kg basis, there appeared to be a dose-response relationship. The median dose amongst responders was significantly higher, at 0.94 mg/kg (0.63-1.92 mg/kg, n = 23), than that observed amongst non-responders, at 0.79 mg/kg (0.64-1.17 mg/kg, n = 21; p=0.045) (see Figure 28). However, this pattern was not evident when comparing those who achieved remission with those who did not. The median dose amongst patients who achieved remission was 0.94 (0.63-1.33, n = 15) versus 0.79 (0.54-1.92, n = 29)(p=0.13).

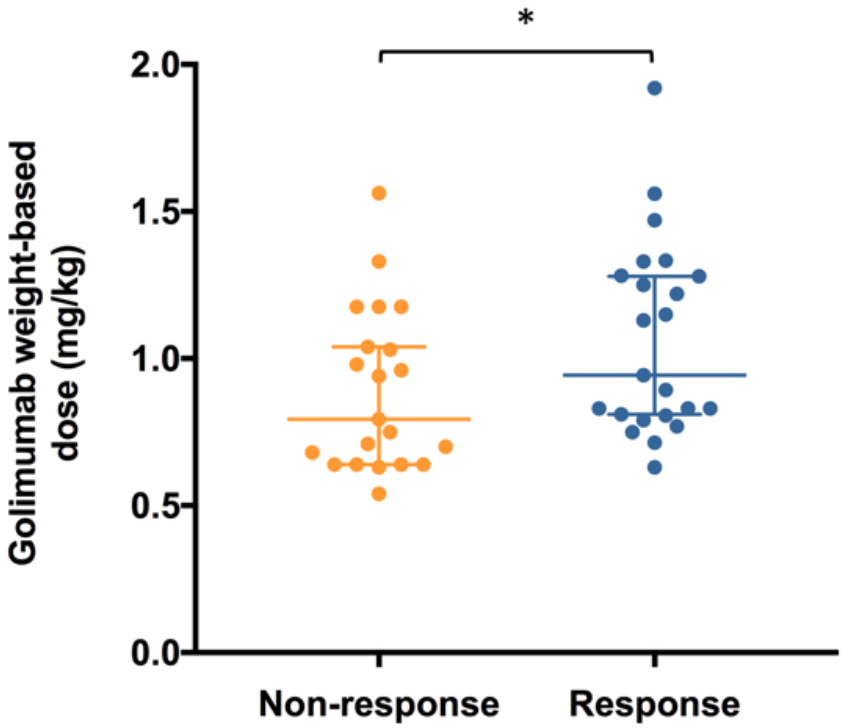



*Figure 28.* Weight-based dose-response analysis demonstrating the individual dose received on a mg/kg basis (median and 95% CI), for responders and non-responders (n = 44, *p=0.045).

**The effect of maintenance dose escalation.**

Amongst our cohort, 25 patients were maintained on 50 mg, 25 patients on 100 mg, and seven patients underwent dose escalation from 50 mg to 100 mg. All maintenance doses were received on a 4-weekly basis (i.e., no patients underwent dose interval shortening). In all cases of dose escalation, the decision was made in view of a clinically suboptimal response as judged by the supervising clinician and may have included information from biochemical or endoscopic disease activity assessments. In 3/7 cases, maintenance dose escalation proved clinically ineffective and treatment was stopped. (However, it should be noted that in one patient FC fell from 2608 to 880 µg/g and in another from 1000 to 368 µg/g). In 2/7 cases, there was a clinically meaningful benefit with both patients achieving remission (both SCCAI 0) and continuing treatment (total durations of 12 and 27 months). In

1/7 there was evidence of clinical and biochemical improvement (SCCAI fell from 19 to 5 and FC fell from 1560 to 171 μg/g) and the patient continues on treatment (total duration 12 months). The final case was recently escalated and has not yet been reassessed but continues on treatment (total duration 4 months).

**Biochemical disease activity.**

Paired pre- and post-induction serum CRP data were available for 43 patients. The median baseline result was 4 (1-59) mg/L and this fell significantly to 2 (1-34) mg/L following induction therapy (p=0.010). The median duration to post-induction CRP measurement was 8 weeks from treatment initiation. Eighteen of the 43 patients (42%) had an elevated CRP (≥5 mg/L) at baseline. Of the 18 patients with an elevated pre-induction CRP, a fall was observed in 16/18 (89%) and normalisation (<5 mg/L) in 11/18 (61%).

Paired pre- and post-induction FC data were available for 20 patients. The median FC fell significantly between from 1096 (15-4800) μg/g to 114 (11-4800) μg/g (p=0.011). The median duration to post-induction FC measurement was 10 weeks from treatment initiation. Eighteen of these 20 patients (90%) had an elevated (≥150 μg/g) FC at baseline. Of the 18 patients with an elevated baseline FC, a fall was observed in 15/18 (83%) and normalisation (<150 μg/g) in 9/18 (50%).

Change in CRP for patients with paired pre- and post-induction data available (n = 43, *p=0.010) is presented in Figure 29. Post-induction scores assessed at a median of 8 weeks following treatment initiation.

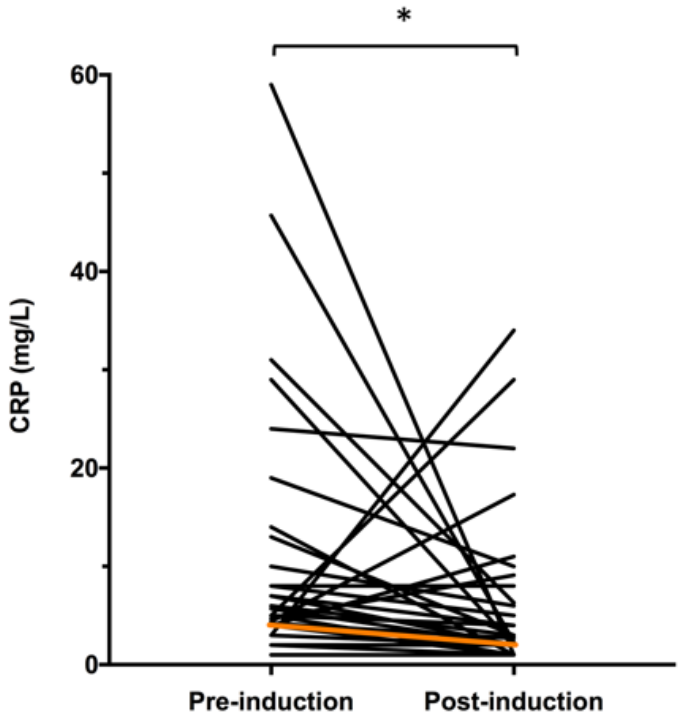



*Figure 29.* Change in CRP for patients with paired pre- and post-induction data available (n = 43, *p=0.010). Post-induction scores assessed at a median of 8 weeks following treatment initiation

Change in FC for patients with paired pre- and post-induction data available (n = 20, *p=0.011) is presented in Figure 30. Post-induction scores assessed at a median of 10 weeks following treatment initiation.

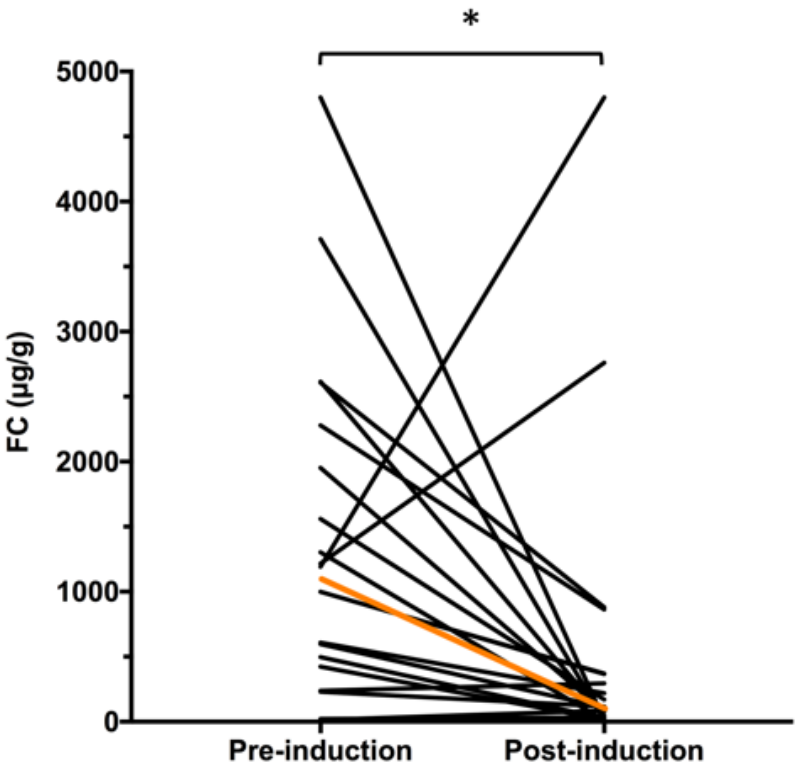



*Figure 30.* Change in FC for patients with paired pre- and post-induction data available (n = 20, *p=0.011). Post-induction scores assessed at a median of 10 weeks following treatment initiation

**Endoscopic outcomes.**

Of our cohort 23/57 (40%) had undergone post-induction endoscopies, whilst still receiving golimumab maintenance treatment and endoscopic Mayo scores were available for all of these. Endoscopic Mayo scores amongst these 23 patients were, Mayo 0: 2 (9%), Mayo 1: 6 (26%), Mayo 2: 6 (26%), Mayo 3: 9 (39%). Using the widely accepted definition for MH of Mayo 0 or 1, results in a MH rate of 8/23 (35%).

**Corticosteroid usage, treatment discontinuation, and surgery.**

At baseline, 27/57 (43%) patients were receiving corticosteroid treatment. This number had fallen to 9/57 (16%) by the time of post-induction clinical follow-up (at a median of 12

weeks from golimumab initiation). Put another way, 18 of the 27 (67%) patients on corticosteroids at baseline were successful in withdrawing from them whilst on golimumab.

A total of 22/57 (39%) patients discontinued treatment with the following reasons, as judged by their supervising physicians: 16 primary non-response, four secondary non-response (loss of response), one patient discontinued due to drug induced rash and one patient was withdrawn from the GO-COLITIS clinical trial due to breach of trial protocol. Four of the patients who failed to respond to golimumab underwent colectomy, resulting in an overall surgery rate of 4/57 (7%). Figure 31 describes the Kaplan-Meier analysis of treatment discontinuation amongst our cohort. The median duration of treatment was 7 (2-28) months. Of the 36 patients who had commenced treatment over 12 months ago, 18 (50%) remained on treatment at one year.

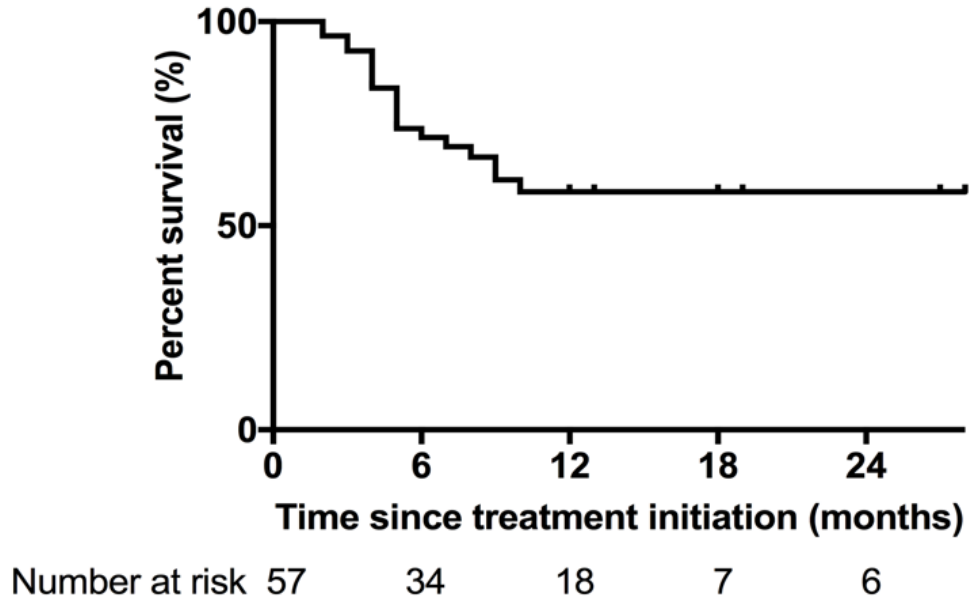



*Figure 31.* Patients remaining on golimumab. Kaplan-Meier analysis illustrating the rate of golimumab continuance

## Discussion

The results of our study closely resemble those seen in large-scale, randomised, placebo-controlled trials (Sandborn et al., 2014a; Sandborn et al., 2014b) as well as previously reported 'real-world' cohorts (Bosca-Watts, 2016; Bressler et al., 2016; Castro-Lariaet al., 2016; Detrez et al., 2016; Hamed et al., 2014; Renna et al., 2016; Taxonera et al., 2016; Tursi et al., 2016a; Tursi et al., 2016b; Varvara et al., 2016). Patients in our cohort had a similar duration of disease and rates of corticosteroid usage to those seen in the PURSUIT trials. However, as previous anti-TNF exposure was an exclusion criterion in PURSUIT, our cohort had a higher rate of prior anti-TNF experience (30%). This finding partly reflects previous patterns of management in the UK, in which anti-TNF agents were not approved for use as maintenance therapy until mid-2015. This resulted in some patients effectively receiving episodic infliximab rescue therapy with a maximum of three induction infusions given during periods of increased disease activity. Of relevance in this context, PURSUIT-M was the first randomised withdrawal study of an anti-TNF in UC, thus, clarifying that induction only is insufficient to maintain a long-term response (Sandborn et al., 2014a). Despite the difference in prior anti-TNF exposure, our observed change in SCCAI (-4) and response rate at a median of 12 weeks (52%) was broadly similar to the change in Mayo score (-2) and response rate at week 6 (51%) seen in PURSUIT-SC (Sandborn et al., 2014b). However, we observed a significantly greater effectiveness, in terms of response, amongst anti-TNF naïve compared with anti-TNF experienced patients. This finding was not replicated in terms of remission, likely due to the relatively smaller numbers of patients achieving this outcome. Our results corroborate a previously reported (abstract) observational study by Taxonera et al. (2016) in which 60% of patients were anti-TNF experienced. Like us, they conducted a

retrospective, multicentre cohort study, and described finding a significant difference in initial response rates based on prior anti-TNF experience. They also reported a numeric difference in longer-term maintained response after 10 months of golimumab, which was observed in 60% of anti-TNF naïve patients but only 39% of those with prior anti-TNF exposure (p=0.15). The most closely corresponding results from our cohort were similar (65% and 23%, respectively, p=0.020). Although the exact reason for anti-TNF cessation in our cohort is not known (prescribing regulations, treatment failure, or adverse effects), this finding is hardly surprising as golimumab is itself an anti-TNF agent and would, therefore, be more likely to fail in cases previously refractory to this mechanism of action. Indeed, this finding has been replicated in other observational cohorts (Castro-Laria et al., 2016; Renna et al., 2016).

An intriguing theme emerging in the use of golimumab is the relationship between exposure and efficacy/effectiveness. This was originally described in PURSUIT-SC with patients in the highest serum golimumab concentration quartiles having greater improvement in median Mayo scores and greater rates of clinical response and clinical remission when compared with those in the lower quartiles (Sandborn et al., 2014b). This pattern was also seen to be true during the maintenance phase (PURSUIT-M) with greater proportions of patients in the higher serum golimumab concentration quartiles achieving clinical response through to week 54, or clinical remission at both weeks 30 and 54, when compared with those in the lower serum concentration quartiles (Sandborn et al., 2014a). Adedokun et al. (2017) reported a rigorous and meticulously performed study of the PK and PD of golimumab using samples taken as part of the PURSUIT trials. As part of these analyses, the authors found SGC to be dose proportional and that a positive correlation exists between concentrations

and efficacy outcomes (clinical response, clinical remission and MH) during induction and maintenance therapy. They then went further by using ROC curve analysis to define SGC that may serve as potential targets for treatment optimisation, proposing thresholds of 2.5 µg/ml at week 6 and 1.4 µg/ml during steady-state maintenance therapy (Adedokun et al., 2017). Prior to this, similar findings were also reported by a group from KU Leuven as part of an observational study of 21 patients being treated with golimumab in a clinical setting. Median golimumab concentrations were significantly higher in partial clinical responders than in non-responders at week 2 (10.0 vs. 7.4 µg/ml, p=0.035) and week 6 (5.1 vs. 2.1 µg/ml, p=0.037). Their ROC curve analysis revealed a cut-off of 2.6 µg/ml at week 6 (90% specificity, 56% sensitivity, AUC 0.79 [95% CI], p=0.034) for the association with a partial clinical response at week 14 (Detrez et al., 2016). Although a commercially available assay was not easily accessible in the UK at the time of our study, we did not involve TDM for this reason; there was, nonetheless, evidence of a dose-response relationship. We observed that patients given a higher dose per kilogram body weight (mg/kg) were more likely to respond than those given a lower dose, calculated on this basis (median 0.94 mg/kg vs. 0.79 mg/kg, p=0.045). With the knowledge that golimumab concentrations are dose proportional, this would appear to offer further observational evidence of an exposure-response relationship. This pattern appeared to be replicated for the achievement of remission but due to the less frequent incidence of this outcome, our results failed to reach statistical significance. Nonetheless, one imagines the same relationship should be true of achieving remission.

Another finding in our study that offers observational evidence for a dose/exposure-response relationship is the benefit derived from maintenance dose escalation in a

proportion of cases. We observed a benefit in three out of six patients who had been dose escalated and subsequently reassessed (with an additional case of dose escalation who has not yet undergone reassessment). This suggests the possibility that the drug is inadequately dosed in some cases of suboptimal response. Indeed, this would fit with the anecdotal experience of patients who sometimes describe deriving an initial benefit from the relatively higher dose, induction regimen before symptoms begin to return during maintenance treatment. Although it should be noted that dose escalation is not within licence or NICE guidance, the 50, and 100 mg maintenance doses are price-matched by the manufacturer and this approach, therefore, incurs no additional costs to healthcare providers. Overall, given the evidence from this study as well as from the others described here, we would encourage clinicians to at least consider this option in patients with a suboptimal response to 50 mg maintenance dosing. Of course, this is not a particularly novel approach in the use of biologics for IBD; evidence-based dose escalation strategies already exist for infliximab (Taxonera et al., 2015), adalimumab (Wolf et al., 2014), vedolizumab (Bruce et al., 2014; Dulai et al., 2016) and seem likely for ustekinumab (based on exposure-response data (Battat et al., 2016). However, empirical dose escalation can incur undue costs (Black et al., 2016; Steenholdt et al., 2014; Velayos et al., 2013) and perhaps a more rational approach to dose optimisation would be to utilise TDM to quantify SGC as well as to identify the presence or absence of ADAb (Vande Casteele & Khanna, 2017). Indeed, several studies are currently planned or underway to further clarify therapeutic thresholds and validate commercially available golimumab assays; GO-KINETIC (GO-KINETIC, no date) at the Academic Medical Centre, Amsterdam, MORE (Drabik et al., 2016) carried out by the German IBD Study Group and GO-LEVEL (GO-LEVEL, no date) by our own group at GSTT (reported in subsequent chapters).

Our study has several limitations. Most notable are its retrospective design and the subjective nature of the clinical disease activity scores employed. In addition, other than rates of surgery, we did not have systematically collected data regarding adverse events. Our cohort also included some patients without paired SCCAI, FC, or CRP measurements made pre-induction and at post-induction review. This is most readily explained by the outpatient nature of golimumab's self-administration treatment regimen, meaning that clinical disease score and biochemical evaluations are not necessarily made as frequently as for intravenously administered biologics. It is also true that some clinicians preferred to use a 'physicians global assessment' type evaluation of treatment effect in lieu of a formal clinical disease activity index such as the SCCAI, and that endoscopy was preferred to FC for disease reassessment in some cases. Although paired CRP data were available for most patients, we had paired FC data on only a limited number of patients. Even with these limited data, FC seemed to outperform CRP in terms of indicating disease activity (elevated in 90%, compared to 42% with an elevated CRP at baseline), although both appeared responsive to the change in disease activity induced by golimumab (an elevated CRP fell in 89% and an elevated FC fell in 83%). Our endoscopic data may also be subject to a negative selection bias based on the premise that most clinicians are more likely to repeat endoscopy in patients with a suboptimal response. Despite this, the proportion we observed to achieve MH (Mayo 0 or 1) was similar to PURSUIT-SC (35% vs. 42%, respectively), as was the proportion achieving complete mucosal normalisation (Mayo 0: 9% vs. 8.3%, respectively). To compensate for missing treatment outcome data, we analysed treatment continuance (as a proxy marker of response) and found that half of our patients remained on golimumab after a year. This finding is not dissimilar to that observed by Bressler et al. (2016) in their retrospective study of 136 golimumab-treated UC patients, in which a one-year continuance

rate of 63% was described. Nonetheless, despite the above-described limitations, we believe the results generated in our study are relevant, reliable, and generalisable. Indeed, this type of observational effectiveness research is becoming increasingly recognised as significant and necessary (Salleron et al., 2016).

Our own previously reported experience of vedolizumab for UC demonstrated similar effectiveness to those described here for golimumab (response rates of 55% and 52%, respectively and remission rates of 39% and 34%, respectively) (Samaan et al., 2016). With NICE approval of vedolizumab coming at a similar time to the approval of anti-TNF agents, clinicians now have a broader range of treatments for UC than ever before. Although certain factors may make vedolizumab preferable (Dart et al., 2017), the choice of mechanism for first line biologic treatment in UC remains a current 'hot-topic' for debate. However, in lieu of clear RCT evidence to settle the matter, our practice is to discuss each case in a multidisciplinary setting. The appropriate choice and management of biologic drugs is often a matter of nuance that incorporates multiple factors, best addressed by physicians, clinical nurse specialists, and pharmacists with a specialist interest in IBD. We, therefore, discuss all relevant aspects of cases where a biologic is being considered as part of a weekly virtual biologics and immunosuppressives clinic (VBIC). This includes IBD-specific factors such as disease activity and the predominance of extra-intestinal manifestations as well as medical comorbidities, such as a predisposition or history of malignancy or infection. In addition, practical factors such as patient preference for route of administration and the management of pressures on infusion suite capacity should be considered. It should be appreciated that this is a rapidly moving field and that the next wave of biologic (e.g., p19

agents) and small molecule (e.g., novel JAK inhibitors and ozanimod) drugs will add further complexity.

# Conclusion

Our early experience with golimumab in two tertiary IBD centres mirrors the effectiveness observed in other real-world cohorts as well as the efficacy demonstrated by the PURSUIT trial programme. Our data demonstrate a clear benefit in terms of symptom control and improvement of objective markers of disease activity, as well as a steroid sparing effect. It also offers further evidence of the dose-response relationship associated with the use of golimumab.

## Significance of the Study

Significance of the study is summarised in Table 27.

**Table 27**
*Significance of the study*

| |
|---|
| **What is already known about this subject?**<br>• The PURSUIT trial programme (an integrated phase 2 and 3 randomised controlled trial) demonstrated the efficacy of golimumab for both the induction and maintenance of remission in moderate-to-severe UC<br>• An exposure-response relationship was observed in a post-hoc analysis of samples collected as part of the PURSUIT trial programme. |
| **What this study adds?**<br>• Our data demonstrate the effectiveness of golimumab in controlling symptoms and improving objective markers of disease activity in a 'real-world' cohort of patients with UC.<br>• Our findings support a dose-response relationship when golimumab is used in clinical practice. |
| **How might it impact on clinical practice in the foreseeable future?**<br>• Clinicians should consider golimumab in patients with UC who are failing conventional therapies as well as in those who are steroid dependent.<br>• In cases of suboptimal response to golimumab 50 mg, 4-weekly maintenance therapy, clinicians may consider dose escalation to 100 mg, 4-weekly administration. It should be understood that this regimen is unlicensed in patients weighing less than 80 kg. |

# Chapter 4: Study of Golimumab Exposure-Response Relationship Using Serum Trough Levels (GO-LEVEL): *Induction*

## Background

Golimumab is a transgenic, fully human monoclonal immunoglobulin G1 antibody that is synthesised from TNF-immunised transgenic mice expressing human immunoglobulin G. The PURSUIT trial programme was a series of randomised, double-blind, placebo-controlled studies that led to regulatory approval for the use of golimumab in UC (Sandborn et al., 2014a; Sandborn et al., 2014b). The comprehensive trial programme consisted of investigation of the most appropriate route of administration (subcutaneous or intravenous (Rutgeerts et al., 2015 ), a phase II dose-ranging study and a phase III trial of induction and maintenance therapy. Subcutaneous administration was found to result in equivalent efficacy and a preferable PK profile when compared with intravenous dosing and is therefore the approved route of administration. PURSUIT-SC demonstrated that induction therapy with golimumab resulted in a significantly greater proportion of patients achieving a clinical response, clinical remission and MH at week 6 compared with placebo (Sandborn et al., 2014b). All subjects from the PURSUIT-SC study were eligible for enrolment into PURSUIT-M, which evaluated the efficacy and safety of golimumab maintenance therapy over 54 weeks (Sandborn et al., 2014a). Ongoing treatment with golimumab was shown to result in a significantly increased rate of sustained clinical benefit (both response and remission) compared with placebo.

In PURSUIT-SC the change from baseline Mayo score and rates of clinical response and clinical remission at week 6 increased with increasing quartiles of SGC (Sandborn et al.,

2014b). Serum concentration quartile analysis of the maintenance trial showed that more patients in the higher quartiles achieved clinical response through to week 54, or clinical remission at both weeks 30 and 54, when compared with those in the lower quartiles (Sandborn et al., 2014a). Subsequent to the original studies, Adedokun et al. (2017) reported a detailed post-hoc study of golimumab's pharmacokinetics and exposure-response using samples taken as part of PURSUIT. A correlation was observed between SGC and efficacy outcomes (clinical response, clinical remission and MH) during both induction and maintenance therapy. Using ROC curve analysis to define SGC thresholds that may serve as potential targets for treatment optimisation resulted in their proposing thresholds of 2.5 ug/ml at week 6 and 1.4 ug/ml during steady-state maintenance therapy (Adedokun et al., 2017). Prior to this, similar findings were also reported by a group from KU Leuven as part of an observational study of 21 patients being treated with golimumab in a clinical setting. Median golimumab concentrations were significantly higher in partial clinical responders than in non-responders at week 2 (10.0 vs. 7.4 ug/ml, p=0.035) and week 6 (5.1 vs. 2.1 ug/ml, p=0.037). Their ROC curve analysis revealed a cut-off of 2.6 µg/ml at week 6 (90% specificity, 56% sensitivity, AUC 0.79 [95% CI], p=0.034) for the association with a partial clinical response after 14 weeks of treatment (Detrez et al., 2016). The authors of both of these studies highlighted the need for further prospective trials to validate their findings and add further validation to commercially available assays for the measurement of SGCs. Data such as these could be used to optimise the use of golimumab in clinical practice and inform prospective TDM trials employing TL to drive dosing.

ADAb were also detected in a small minority of patients (2.9%) in the PURSUIT trials and the majority of these (67.7%) were described as neutralising. Their occurrence was significantly

less common in patients who were receiving concomitant immunomodulators (1.1%) compared with patients who were not (3.8%). However, due to the low observed incidence it is difficult to draw conclusions regarding their impact on efficacy. Nonetheless, a clearer understanding of their impact on drug exposure and subsequently, disease activity would be of benefit in defining the optimal use and monitoring of golimumab.

Despite the fact that the PURSUIT trial programme yielded positive results and met its primary endpoints, unanswered questions remain regarding the optimal use of golimumab in UC. For example, how could the observed rates of primary and secondary non-response (approximately 50% and 40%, respectively) be minimised? In addition to significant rates of non-response, the majority of patients who did respond to the drug remained symptomatic to some degree, were on concomitant steroids, and were without a 'normal or inactive' (endoscopic Mayo 0) mucosal appearance (Hanauer, 2014). It is possible that given a more detailed understanding of golimumab's PK and exposure-response relationship, these outcomes could be improved upon. Most important of these is further evidence that can be used to quantify a minimum exposure threshold that results in clinical benefit. We, therefore, designed and conducted a study of the **GO**limumab exposure-response relationship using serum TL (GO-LEVEL) to add to the growing body of evidence in this regard. GO-LEVEL was an open-label, phase IV, investigator initiated study which included a prospective cohort of UC patients commencing golimumab induction therapy (described in this chapter), as well as a cross-sectional cohort receiving maintenance treatment (described in Chapter 5: Study of Golimumab Exposure-Response Relationship Using Serum Trough Levels (*GO-LEVEL): Maintenance*).

Included as part of the clinical disease activity assessments made in GO-LEVEL was a patient reported outcome (PRO). PROs have an increasingly important role in IBD and this is true of monitoring of disease activity in clinical practice (van Deen, Esrailian, & Hommes, 2015) and as well as the evaluation of new therapies, when used as clinical trial endpoints (Williet, Sandborn, & Peyrin-Biroulet, 2014). An interim two-item PRO (PRO2) has been developed for UC that ranges from 0-6 and consists of the patient derived items from the Mayo score (rectal bleeding and stool frequency) (Jairath, Khanna, & Zou, 2015). PRO2 was internally validated against endoscopic outcomes. By including it in our study evaluations, we aimed to add external validation across a range of clinical, biochemical, and QoL outcomes.

### Aims

The primary aim of the GO-LEVEL induction cohort was to define a week 6 golimumab TL concentration that predicts response at week 14. Secondary aims were to define golimumab TL concentrations at weeks 6, 10, and 14 that predict response at each time point, respectively. Tertiary aims included investigation of the relationship between serum golimumab TL and clinical disease activity (SCCAI and PRO2), biochemical markers of disease activity (CRP and FC) and QoL (evaluated using IBD-Control). The frequency of AGA was also investigated, as well as their relation to TL and disease activity.

## Methods

#### Patients.

Patients were recruited from the gastroenterology department at GSTT. Potential study candidates were identified during the course of standard clinical consultations, at the time of endoscopy and/or during review in our weekly VBIC. Any member of the multidisciplinary

IBD team could identify eligible patients, including registrars, clinical research fellows, consultants, clinical nurse specialists, research nurses, or pharmacists. To increase recruitment, participants could also be identified at local patient identification centres (PIC). A PIC is a site where participants are identified and referred to a different centre (in this case GSTT) specifically to take part in a research study. Both Queen Elizabeth Hospital Woolwich and University Hospital Lewisham were recruited as PICs, including arrangement of the necessary regulatory approvals and research referral pathways.

For each patient recruited the decision to commence biologic was taken within the context of NICE guidance as well as a locally agreed IBD treatment pathway (NHS, 2017). These recommend biologic treatment for adult patients with moderate-to-severe UC, who have had an inadequate response to, or are unable to tolerate, one or more of the following conventional therapies: oral 5-aminosalicylates, oral corticosteroids, immunomodulators; or who are corticosteroid dependent. The study inclusion criteria for the GO-LEVEL induction cohort were as follows:

- Aged 18 years or over
- Able to provide written informed consent to participate
- Moderate-to-severe UC, defined as:
    - SCCAI >5 *and,*
        - A raised FC (>59 µg/g) ***or,***
        - A raised CRP (>5 mg/L) ***or,***
        - Endoscopic disease activity Mayo 2 or above.

***Evaluated within 4 weeks of screening***

- VBIC recommendation to commence golimumab
- Sufficient English language skills to understand the patient information sheet and consent form

Patients were excluded from participation if they fulfilled any of the following criteria:

- Contra-indication to golimumab: tuberculosis, severe infections, or congestive cardiac failure
- Imminent need for colectomy (i.e., colectomy was being planned)
- Previous primary non-response to anti-TNF therapy in the opinion of the investigator
- Previous treatment with more than one anti-TNF therapy (excluding golimumab)

Demographic information as well as the following disease-related data were collected: disease duration, distribution using the Montreal classification (Satsangi et al., 2006), BMI, prior anti-TNF exposure, concomitant immunomodulation, and corticosteroids.

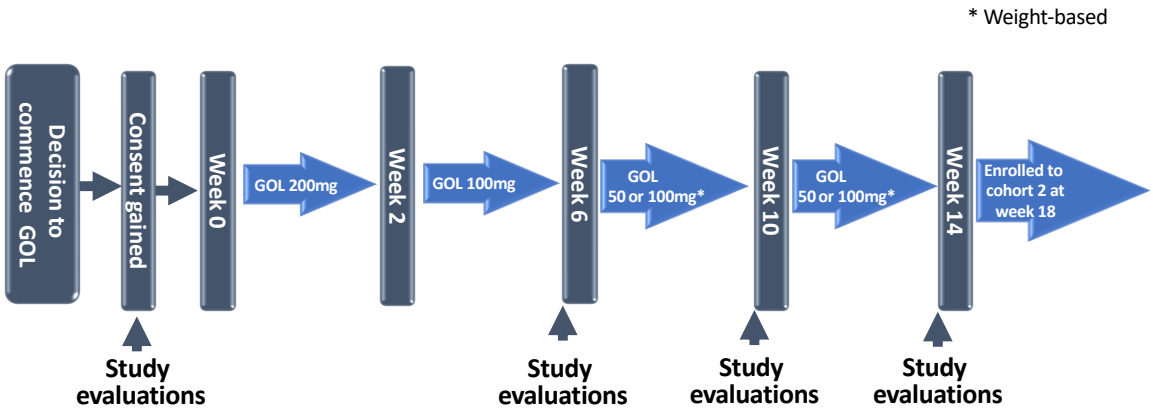
**Golimumab dosing.**

All patients received subcutaneously administered golimumab induction dosing of 200 mg at week 0 and 100 mg at week 2. As per the original approved dosing strategy, from week 6 onwards the default dose for patients with body weight ≥80 kg was 100 mg, and for those with body weight <80 kg was 50 mg, every four weeks. However, more in line with the updated dosing strategy (Philip et al., 2019) and our department's standard of care (Samaan et al., 2018b), patients with weight <80 kg with a suboptimal clinical and/or biochemical response to 50 mg were escalated to 100 mg every four weeks. Decisions regarding dose

escalation were made as part of a weekly multidisciplinary, biologics focused IBD meeting and without knowledge of TDM as samples were 'batched' and analysed at a later date.

**Study evaluations.**

Study evaluations were carried out at baseline and after six, 10, and 14 weeks of treatment. Each study visit included assessments of clinical disease activity, QoL, and biochemical activity. Assessments at weeks 6, 10, and 14 were arranged to coincide with TL measurements, here defined as within 4 days of the subsequent dose (see Figure 32 and Table 28).




**Study evaluations**

| | |
|---|---|
| Blood sample: | Golimumab trough level and anti-drug antibodies, CRP, albumin |
| Stool sample: | Faecal calprotectin |
| Disease activity indices: | SCCAI, PRO2 |
| Quality of life indices: | IBD-control-8, IBD-Control-VAS |

*Figure 32.* Study evaluations during the GO-LEVEL induction study

**Table 28**

*GO-LEVEL induction study flow chart*

| Screen Visit (day) | -90 to day 0 | 0 | 14 | 38 to 42 | 42 | 66 to 70 | 70 | 94 to 98 | 98 |
|---|---|---|---|---|---|---|---|---|---|
| **Week** | | 0 | 2 | | 6 | | 10 | | 14 |
| **Signed Informed consent** | X | | | | | | | | |
| **Collection of demographic and UC disease-related data** | X | | | | | | | | |
| **Review inclusion/exclusion criteria** | X | | | | | | | | |
| **Golimumab administration (self-administered by patients)** | | X | X | | X | | X | | X |
| **Serum golimumab concentration and antidrug antibody measurements** | | | | X | | X | | X | |
| **Clinical disease activity scores (SCCAI and PRO2)** | X | | | X | | X | | X | |
| **Injection site reaction and IBD-relevant concomitant medication review** | | | | X | | X | | X | |
| **Serum CRP and albumin measurements** | X | | | X | | X | | X | |
| **FC** | X | | | X | | X | | X | |
| **QoL assessment (IBD-Control)** | X | | | X | | X | | X | |

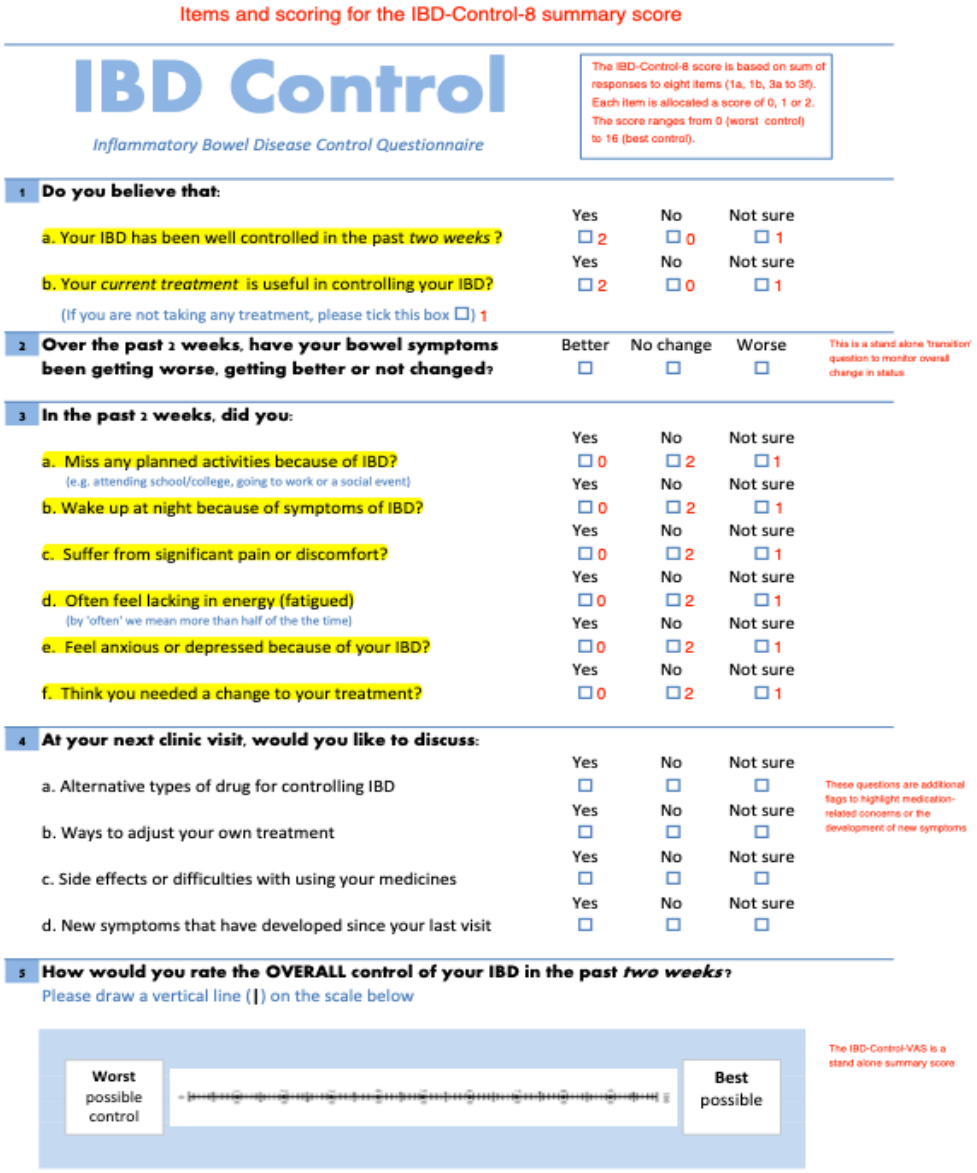
***Clinical disease activity.***

Clinical disease activity was primarily evaluated using the SCCAI, which includes bowel frequency (day and night), urgency, rectal bleeding, general wellbeing and extracolonic

features (Walmsley et al., 1998). It ranges from 0 to 19, with higher values indicating increasing symptom severity. Clinical remission was defined as an SCCAI ≤2 and response as an SCCAI ≤5, with a decrease by ≥2 (Higgins et al., 2005). In addition to SCCAI, a novel interim PRO for UC, PRO2, was recorded alongside SCCAI. PRO2 ranges from 0 to 5 and includes bowel frequency and rectal bleeding (Jairath et al., 2015). To provide a conservative estimate of treatment efficacy, non-responder imputation analysis was used to deal with missing data for treatment outcome analyses. For PK analyses patients with missing disease activity or TDM data were simply excluded from analysis at that time point.

***Quality of life.***

QoL was evaluated using the IBD-Control-8 index, which also comprises a 100 mm visual analogue scale (VAS). This patient completed questionnaire has been shown to show strong validity versus more complex QoL questionnaires (UK-IBD-Q), generic utility measures, disease activity scores, and global physician assessment (Bodger et al., 2014). The entire IBD-Control questionnaire is shown in Figure 33 but only the highlighted questions are included in the IBD-Control-8 score. This ranges from 0 to 16 with higher scores indicating better QoL. Higher scores on the VAS (ranging 0-100) also indicate better QoL.


Items and scoring for the IBD-Control-8 summary score

IBD Control

*Inflammatory Bowel Disease Control Questionnaire*

The IBD-Control-8 score is based on sum of responses to eight items (1a, 1b, 3a to 3f). Each item is allocated a score of 0, 1 or 2. The score ranges from 0 (worst control) to 16 (best control).

**1 Do you believe that:**

| | Yes | No | Not sure |
|---|---|---|---|
| a. Your IBD has been well controlled in the past *two weeks*? | ☐ 2 | ☐ 0 | ☐ 1 |
| b. Your *current treatment* is useful in controlling your IBD? | ☐ 2 | ☐ 0 | ☐ 1 |

(If you are not taking any treatment, please tick this box ☐) 1

**2 Over the past 2 weeks, have your bowel symptoms been getting worse, getting better or not changed?**

| Better | No change | Worse |
|---|---|---|
| ☐ | ☐ | ☐ |

This is a stand alone 'transition' question to monitor overall change in status

**3 In the past 2 weeks, did you:**

| | Yes | No | Not sure |
|---|---|---|---|
| a. Miss any planned activities because of IBD? (e.g. attending school/college, going to work or a social event) | ☐ 0 | ☐ 2 | ☐ 1 |
| b. Wake up at night because of symptoms of IBD? | ☐ 0 | ☐ 2 | ☐ 1 |
| c. Suffer from significant pain or discomfort? | ☐ 0 | ☐ 2 | ☐ 1 |
| d. Often feel lacking in energy (fatigued) (by 'often' we mean more than half of the the time) | ☐ 0 | ☐ 2 | ☐ 1 |
| e. Feel anxious or depressed because of your IBD? | ☐ 0 | ☐ 2 | ☐ 1 |
| f. Think you needed a change to your treatment? | ☐ 0 | ☐ 2 | ☐ 1 |

**4 At your next clinic visit, would you like to discuss:**

| | Yes | No | Not sure |
|---|---|---|---|
| a. Alternative types of drug for controlling IBD | ☐ | ☐ | ☐ |
| b. Ways to adjust your own treatment | ☐ | ☐ | ☐ |
| c. Side effects or difficulties with using your medicines | ☐ | ☐ | ☐ |
| d. New symptoms that have developed since your last visit | ☐ | ☐ | ☐ |

These questions are additional flags to highlight medication-related concerns or the development of new symptoms

**5 How would you rate the OVERALL control of your IBD in the past *two weeks*?**

Please draw a vertical line (|) on the scale below

Worst possible control — Best possible

The IBD-Control-VAS is a stand alone summary score

*Figure 33.* IBD-Control questionnaire. Only the highlighted questions are included in the IBD-Control-8 score

***Biochemical disease activity.***

Biochemical disease activity was evaluated using serum CRP and FC measurements. FC was measured in the Viapath laboratory at KCH, using the fCal assay (Bühlmann, Switzerland). Stool samples were kept at 4°C until transfer to KCH. Following extraction of calprotectin from stools, samples were stored at -80°C until quantification. Biochemical remission was

defined as FC <250 ug/g. Combined clinical-biochemical remission was defined as SCCAI ≤2 *and* FC <250 ug/g.

***Measurement of SGC and AGA concentrations***

Samples for measurement of SGC and AGA concentrations were collected prior to injections at weeks 6, 10, and 14. Samples were processed according to the instructions provided by the manufacturers, using the commercially available LISA TRACKER ELISA (Theradiag, France). This assay is drug-sensitive and therefore, is only able to detect ADAb when drug levels are low or absent. AGA were considered present at titres ≥10 ng/ml.

## Statistics

### Sample size.

To achieve a power of 80%, with two-sided significance, and to detect a mean difference in serum concentration of 2 mg/L between responders and non-responders, a sample size of approximately 42 patients was considered sufficient based on existing data (Sandborn et al., 2014a; Sandborn et al., 2014b).

### Statistical Analysis.

Continuous data are summarised as medians and range (in brackets). Paired SCCAI, CRP, and FC values were compared using the Wilcoxon signed-rank test. Categorical variables were compared using the Fisher's exact or Mann-Whitney U (GraphPad Prism v8.2.1). Correlations between variables were calculated with the Spearman correlation coefficient ($r_s$). ROC curve analysis was used to identify target SGC thresholds. The Chi-squared test for trend (also known as the Cochrane-Armitage test for trend) was used to analyse SGC quartile data. Unless stated, p values are non-significant. All data below/above the limit of

quantification were substituted with the value of the lower/upper limit of quantification, i.e., CRP 1 mg/L for levels of <1 mg/L, and FC 4800 µg/g for levels >4800 µg. Analyses were based on the full analysis set, defined as all subjects who had received golimumab for the entire 14-week study period and had at least one valid post-baseline assessment for the outcome variables of interest.

## Ethical & Regulatory Approval

This study was granted approval by the Medicines and Healthcare products Regulatory Authority (MHRA), NHS Health Research Authority (HRA) and our institute's Gastroenterology Research Project Review Board. It was registered with ClinTrials.gov (NCT03124121) and EudraCT (2017-001374-42).

# Results

### Patient characteristics.

Recruitment to the GO-LEVEL induction cohort commenced in September 2017 and was completed in March 2019, with the final patient undergoing their final study visit in May 2019. A total of 42 patients commencing golimumab induction therapy were recruited; 39 completed the 14-week study protocol (see Table 29). Two patients discontinued due to lack of efficacy and one discontinued treatment due to a serious adverse event (SAE: facet joint infection). They were therefore excluded from the PK analyses but were included in treatment outcomes as non-responders.

In addition to clinically active disease, 30 patients had objective confirmation of disease activity based on endoscopy and the remaining nine based on an elevated FC.

**Table 29**

*Characteristics of patients included in pharmacokinetic analyses (UCEIS, ulcerative colitis endoscopic index of severity)*

| **Characteristics of patients included in PK analyses** | **n = 39** |
|---|---|
| **Gender, male: female, n (%)** | 22:17 (56:44) |
| **Median age (range), years** | 37 (24-48) |
| **Median disease duration (range), years** | 7 (0.5-28) |
| **Median BMI (range)** | 24.3 (17.9-39.0) |
| **Median Mayo endoscopic score (range), n=30** | 2 (2-3) |
| **Median UCEIS (range), n=30** | 4 (3-8) |
| **Median FC (range), ug/g, n=39** | 426 (5-5420) |
| **Disease extent, n (%)** | |
| ***Proctitis*** | 3 (8) |
| ***Left-sided*** | 20 (51) |
| ***Extensive*** | 16 (41) |
| **Concomitant immunomodulation, n (%)** | |
| ***Thiopurine*** | 20 (51) |
| ***Methotrexate*** | 3 (8) |
| **Corticosteroids, n (%)** | 17 (44) |
| **Prior anti-TNF experience, n (%)** | |
| ***Naïve*** | 37 (95) |
| ***Exposed*** | 2 (5) |

**Predictive value of serum golimumab concentrations.**

The predictive value of week 6 and 10 SGC with regards to week 14 outcomes was investigated (see Table 30). This showed no significant differences in SGC at weeks 6 or 10 between patients achieving combined clinical-biochemical remission at week 14.

**Table 30**
*Predictive value of week 6 and 10 SGC on week 14 combined clinical-biochemical remission*

| | Median SGC (range), ug/ml | |
|---|---|---|
| | **Week 6** | **Week 10** |
| **Combined clinical-biochemical remission at week 14** | 3.7 | 3.4 |
| **Not in combined clinical-biochemical remission at week 14** | 3.2 | 2.3 |
| ***p*-value** | 0.88 | 0.10 |

*Correlations between SGC, disease activity, and QoL*

Correlations between week 6 SGC and outcomes at weeks 6, 10, and 14 were analysed using the Spearman correlation coefficient ($r_s$). An inverse correlation was observed between week 6 SGC and concurrent clinical disease activity but only when measured using PRO2 ($r_s$ = -0.36, p=0.03) rather than SCCAI ($r_s$ = -0.25, p=0.15). Similarly, inverse correlations were seen with week 6 biochemical disease activity, measured by both CRP ($r_s$ = -0.43, p=0.01) and FC ($r_s$ = -0.37, p=0.03). No other significant correlations were observed between week 6 SGC and other concurrent evaluations. Week 6 SGC were also observed to correlate with week 14 CRP ($r_s$ = -0.34, p=0.04) and albumin ($r_s$ = 0.36, p=0.04) values. However, no other significant correlations were seen with disease activity evaluations made at baseline, weeks

10, or 14. Week 14 SGC were observed to correlate with FC measurements made at that time point ($r_s$ = -0.34, p=0.04) but not with other concurrent evaluations (see Table 31).

**Table 31**

*Correlations between week 6 serum golimumab concentrations and clinical, biochemical and quality of life outcomes at baseline, weeks 6, 10 and 14*

| | | | | Week 6 SGC | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Baseline | | Week 6 outcomes | | Week 10 outcomes | | Week 14 outcomes | |
| **Outcomes/biomarkers** | | **$r_s$** | ***p*** | **$r_s$** | ***p*** | **$r_s$** | ***p*** | **$r_s$** | ***p*** |
| **Clinical disease activity, median (range)** | **SCCAI** | 0.02 | 0.89 | -0.25 | 0.15 | -0.10 | 0.56 | -0.03 | 0.88 |
| | **PRO2** | 0.15 | 0.39 | -0.36 | **0.03** | -0.25 | 0.16 | -0.14 | 0.42 |
| **QoL, median (range)** | **IBD-Control-8** | -0.08 | 0.62 | 0.19 | 0.26 | 0.28 | 0.11 | 0.24 | 0.17 |
| | **IBD-Control-VAS** | 0.08 | 0.64 | 0.03 | 0.85 | 0.049 | 0.78 | 0.02 | 0.89 |
| **Biochemical disease activity, median (range)** | **CRP** | -0.16 | 0.36 | -0.43 | **0.01** | -0.16 | 0.36 | -0.34 | **0.04** |
| | **FC** | 0.31 | 0.06 | -0.37 | **0.03** | 0.019 | 0.92 | -0.23 | 0.23 |
| | **Albumin** | -0.02 | 0.90 | 0.28 | 0.11 | 0.31 | 0.08 | 0.36 | **0.04** |

**Table 32**

*Correlations between week 14 serum golimumab concentrations and clinical, biochemical and quality of life outcomes*

| Outcomes/biomarkers (n), week 14 | | Week 14 SGC $r_s$ | Week 14 SGC p-value |
|---|---|---|---|
| **Clinical disease activity, median (range)** | **SCCAI** | -0.23 | 0.19 |
| | **PRO2** | -0.25 | 0.15 |
| **QoL, median (range)** | **IBD-Control-8** | 0.30 | 0.07 |
| | **IBD-Control-VAS** | 0.17 | 0.33 |
| **Biochemical disease activity, median (range)** | **CRP** | -0.29 | 0.09 |
| | **FC** | -0.34 | **0.04** |
| | **Albumin** | 0.30 | 0.08 |

**Pharmacokinetics.**

Amongst the 39 patients, a total of 106 golimumab TDM samples were taken across the three study time points during the 14-week protocol (11 samples were omitted due to missed study visits or incorrect timing of administration e.g., prior to the visit). The median SGC at week 6 was 3.3 ug/ml (1.3-8.0) and samples were taken at a median of 1.5 days (0-4 days) prior to the subsequent administration. Although there was a trend towards week 6 clinical responders having higher concentrations than non-responders, the difference failed to reach statistical significance (4.7 ug/ml vs. 3.0 ug/ml, p=0.09). However, a significant difference in median SGC was seen when comparing patients achieving clinical remission at week 6 with those who did not (4.8 ug/ml vs. 3.0 ug/ml, p=0.02). Similarly, a significant

difference was observed between those achieving combined clinical-biochemical remission (5.0 ug/ml) and those who did not (3.0 ug/ml, p=0.02).

The median SGC at week 10 was 2.7 ug/ml (0.5-6.6) and samples were taken at a median of 0 days (0-4 days) prior to the subsequent administration (i.e., on the same day). There were no differences between SGC in clinical responders and non-responders (2.8 ug/ml vs. 2.6 ug/ml, respectively, p=0.38), clinical remitters and non-remitters (2.5 ug/ml vs. 2.7 ug/ml, respectively, p=0.77), or combined clinical-biochemical remitters and non-remitters (2.5 ug/ml vs. 3.4 ug/ml, respectively, p=0.42).

Similar results were observed at week 14, when the median SGC was 2.1 ug/ml (0.6-5.4) and samples were taken at a median of 0 days (0-4 days) prior to the subsequent administration (i.e., on the same day). Similar to week 10, no significant differences were found between outcome subgroups, however defined: clinical responders versus non-responders (2.1 ug/ml vs. 1.9 ug/ml, respectively, p=0.27), clinical remitters vs. non-remitters (2.2 ug/ml vs. 1.8 ug/ml, respectively, p=0.13), combined clinical-biochemical remitters versus non-remitters (2.4 ug/ml vs. 1.8 ug/ml, respectively, p=0.08).

The use of concomitant immunosuppressive medication did not appear to have an effect on SGC at any of the time points studied. The median SGC amongst patients on immunosuppressants at weeks 6, 10, and 14 were 3.2, 2.5 and 1.9 ug/ml compared with 4.0, 3.3 and 2.1 ug/ml in those who were not (p=0.38, 0.39 and 0.79, respectively) (see **Error! Reference source not found.**).

**Table 33**

*Median serum golimumab concentrations amongst patients who achieved a clinical response, clinical remission and combined clinical-biochemical remission compared to those who did not at weeks 6, 10 and 14*

| | | Median SGC, µg/ml | | |
|---|---|---|---|---|
| | | **Week 6** | **Week 10** | **Week 14** |
| **Clinical response** | Achieved | 4.7 | 2.8 | 2.1 |
| | Not achieved | 3.0 | 2.6 | 1.9 |
| | p-value | 0.09 | 0.38 | 0.27 |
| **Clinical remission** | Achieved | 4.8 | 2.5 | 2.2 |
| | Not achieved | 3.0 | 2.7 | 1.8 |
| | p-value | 0.02 | 0.77 | 0.13 |
| **Combined clinical-biochemical remission** | Achieved | 5.0 | 2.5 | 2.4 |
| | Not achieved | 3.0 | 3.4 | 1.8 |
| | p-value | 0.02 | 0.42 | 0.08 |

**Quartile analysis.**

To further investigate the exposure-response relationship, quartile analysis was carried out by dividing the cohort into four groups depending on week 6 SGC (<2.5 ug/ml, 2.5-<3.8 ug/ml 3.8-<5.0, and ≥5.0 ug/ml). These analyses all demonstrated significant trends for patients with higher exposure experiencing better outcomes, including clinical response, clinical remission, and combined clinical-biochemical remission. Rates of clinical response were 66%, 56%, 78%, and 100% for the first, second, third, and fourth quartiles, respectively

(p=0.046). Corresponding rates of clinical remission were 33%, 44%, 56%, and 90% (p=0.01), respectively. The trend appeared even more evident for rates of combined clinical-biochemical remission; 22%, 22%, 33%, and 78% (p=0.01), respectively (see **Error! Reference source not found.**).

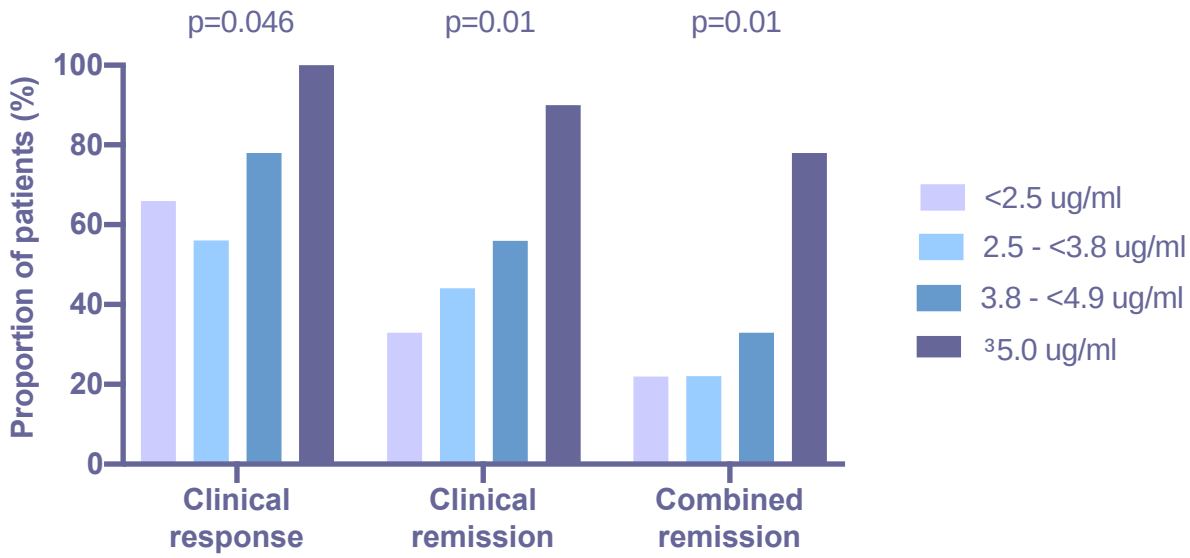



*Figure 34.* Proportion of patients in clinical response, clinical remission and combined remission according to SGC quartile at week 6

**Identification of a target threshold.**

ROC curve analysis was used to identify an SGC that most closely associates with clinical response, clinical remission, and combined clinical-biochemical remission at week 6. An SGC >3.2 ug/ml appeared to be the optimal threshold for the achievement of clinical response, with an area under the ROC curve (AUROC) of 0.69, sensitivity 0.64, and specificity 0.78. For both clinical remission and combined clinical-biochemical remission, the optimal threshold was found to be 3.8 ug/ml. The AUROC for clinical remission was 0.72 (sensitivity 0.67, specificity 0.75) and for combined remission was 0.73 (sensitivity 0.71, specificity 0.68) (see **Error! Reference source not found.**)

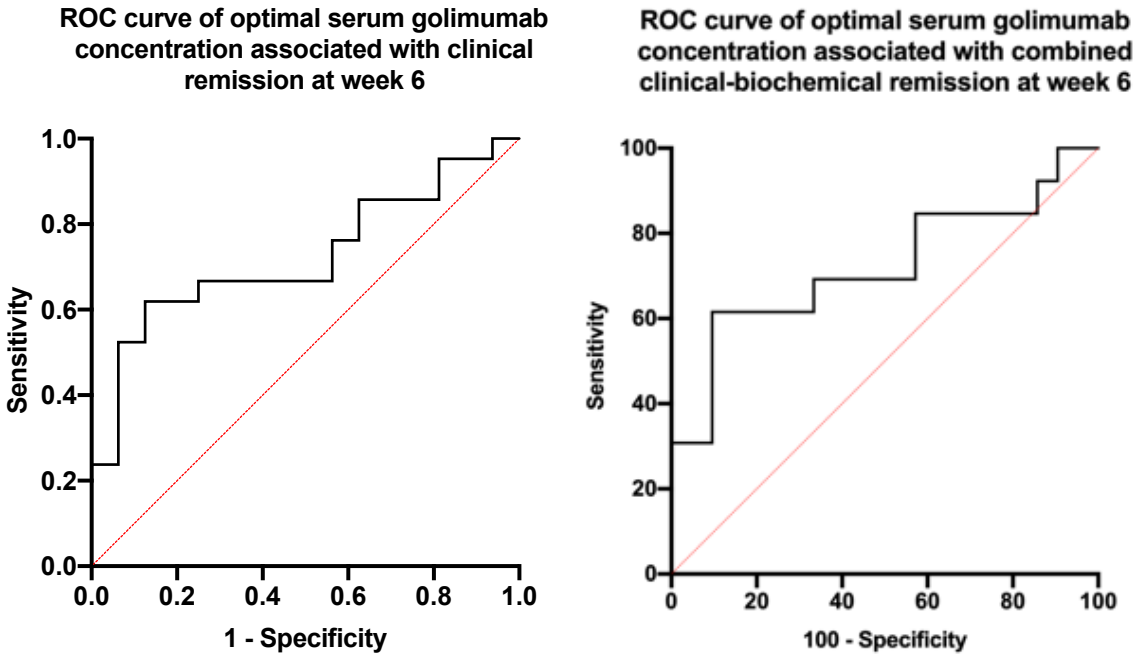



*Figure 35.* ROC analysis of optimal serum golimumab concentration associated with clinical remission (left) and combined clinical-biochemical remission (right) at week 6

**Clinical disease activity.**

By week 6, clinical disease activity had reduced significantly from baseline and remained significantly improved at weeks 10 and 14. This improvement was evident whether activity was judged using SCCAI or PRO2. Median SCCAI values fell from 8 (5-15) at baseline to 2 (0-12, $p<0.0001$) at week 6, 1.5 (0-12, $p<0.0001$) at week 10, and 2 (0-13, $p<0.0001$) at week 14. Corresponding median PRO2 values were 4 (2-6), 0.5 (0-5, $p<0.0001$), 0 (0-5, $p<0.0001$), and 1 (0-5, $p<0.0001$).

Rates of clinical response (SCCAI≤5, with a decrease by ≥2) at weeks 6, 10, and 14 were 29/42 (69%), 28/42 (67%) and 28/42 (67%), respectively. Corresponding rates of clinical remission (SCCAI≤2) were 22/42 (52%), 23/42 (55%), and 22/42 (52%). Rates of combined

clinical-biochemical remission (SCCAI ≤2 and FC <250) at weeks 6, 10, and 14 were 15/42 (36%), 16/42 (38%) and 17/42 (40%), respectively.

**Quality of life.**

By week 6, QoL had improved significantly from baseline and remained significantly improved at weeks 10 and 14. This improvement was evident whether QoL was judged using IBD-Control-8 or the accompanying VAS (IBD-Control-VAS). Median IBD-Control-8 values increased from 3 (0-14) at baseline to 11 (2-16, p<0.0001) at week 6, 12 (0-16, p<0.0001) at week 10, and 12.5 (0-16, p<0.0001) at week 14. Corresponding median IBD-Control-VAS values were 35 (2-80), 64.5 (18-100, p<0.0001), 69.5 (17-100, p<0.0001), and 75 (20-100, p<0.0001).

**Biochemical disease activity.**

FC values fell from a median of 426 ug/g (5-5420) at baseline to 109 ug/g (5-2920, p=0.021) at week 6, 126 ug/g (5-2800, p=0.0022) at week 10,and 46 ug/g (5-2000, p=0.0003) at week 14.

Baseline median CRP was 2 mg/L (1-50) and this fell to 1 mg/L (1-34) by week 6, although this decrease failed to reach statistical significance (p=0.071). It remained 1 mg/L at weeks 10 and 14 but the results at these time points were both significantly lower than baseline (p=0.0078 and p=0.0010, respectively).

Median albumin values increased from 45 g/L (35-51) at baseline to 46 g/L (33-56, p=0.0027) at week 6, 45.5 g/L (33-52, p=0.0046) at week 10, and 46 g/L (33-52, p=0.0013) at week 14 (see Table 34 ).

**Table 34**

*Clinical, biochemical and quality of life outcomes for induction study patients at baseline and weeks 6, 10 and 14*

| Outcome (n = 39) | | Baseline | Week 6 | p-value vs. baseline | Week 10 | p-value vs. baseline | Week 14 | *p*-value vs. baseline |
|---|---|---|---|---|---|---|---|---|
| **Clinical disease activity, median (range)** | **SCCAI** | 8 (5-15) | 2 (0-12) | **<0.0001** | 1.5 (0-12) | **<0.0001** | 2 (0-13) | **<0.0001** |
| | **PRO2** | 4 (2-6) | 0.5 (0-5) | **<0.0001** | 0 (0-5) | **<0.0001** | 1 (0-5) | **<0.0001** |
| **QoL, median (range)** | **IBD-Control-8** | 3 (0-14) | 11 (2-16) | **<0.0001** | 12 (0-16) | **<0.0001** | 12.5 (0-16) | **<0.0001** |
| | **IBD-Control-VAS** | 35 (2-80) | 64.5 (18-100) | **<0.0001** | 69.5 (17-100) | **<0.0001** | 75 (20-100) | **<0.0001** |
| **Biochemical disease activity, median (range)** | **CRP, mg/L** | 2 (1-50) | 1 (1-34) | 0.071 | 1 (1-33) | **0.0078** | 1 (1-50) | **0.0010** |
| | **FC, ug/g** | 426 (5-5420) | 109 (5-2920) | **0.021** | 126 (5-2800) | **0.0022** | 46 (5-2000) | **0.0003** |
| | **Albumin, g/L** | 45 (35-51) | 46 (33-56) | **0.0027** | 45.5 (33-52) | **0.0046** | 46 (33-52) | **0.0013** |

**Golimumab dosing.**

Of the 39 patients in the induction cohort, 11 weighed over 80 kg, and 28 weighed under 80 kg. Of the 28 under 80 kg, 9 (32%) underwent dose escalation from 50 mg to 100 mg every 4 weeks during the 14-week study protocol. Four of these were escalated from week 6 (i.e., from the beginning of weight-based dosing), one additional patient was escalated at week 10 and the final six were escalated at week 14 (study exit). The remaining 19 (68%) patients weighing under 80 kg remained on the standard (licensed) dosing of 50 mg every four weeks. The decision to dose escalate was made in a multidisciplinary setting on the basis of standard clinical and biochemical markers but without any information regarding TDM.

The median week 14 SGC in five patients escalated at weeks 6 or 10 did not differ significantly from patients under 80 kg who continued on standard 50 mg dosing (1.9 ug/ml vs. 2.1 ug/ml, respectively, p=0.34). Neither did it differ from the median SGC of the rest of the cohort as a whole (those <80 kg on 50mg as well as those ≥80 kg on 100 mg) at 2.1 ug/ml (p=0.27).

**Effect of body weight and BMI.**

We observed a significant inverse correlation between body weight and SGC at week 6 ($r_s$=-0.35, p=0.03) but no significant correlations were evident at weeks 10 ($r_s$=-0.16, p=0.36,) or 14 ($r_s$ = -0.0095, p=0.59). Moreover, based on our suggested week 6 therapeutic threshold of >3.8 ug/ml, patients weighing ≥80 kg were significant less likely to achieve a therapeutic level at this time point than those weighing <80 kg (2/11 vs. 16/28, p=0.04). No significant correlations were observed between baseline BMI and SGC measurements at weeks 6 ($r_s$=-0.20, p=0.23), 10 ($r_s$=-0.07, p=0.71), or 14 ($r_s$=0.06, p=0.74).

**Anti-golimumab antibodies.**

Amongst the cohort of 39 patients who completed the 14-week study protocol, no AGA above a titre of ≥10 ng/ml were detected. However, an AGA titre of 12 ng/ml was observed at week 6 in a patient who withdrew before completing the study protocol. His disease activity increased after the week 6 assessments, failed to respond to rescue infliximab and he consequently underwent a colectomy at week 8 (described below as an SAE). The concurrent SGC was 2.19 ug/ml and on repeat testing, the AGA titre remained positive at 10 ng/ml.

**Safety.**

Four SAEs were observed as part of the GO-LEVEL induction cohort, two of which involved a single patient and one of which was classified as a serious adverse reaction (SAR). One patient with steroid refractory, severely active disease at baseline (Mayo endoscopic score 3), who was commenced on azathioprine and golimumab concurrently developed azathioprine induced pancreatitis. Serum amylase at the time of admission was 641U/L and this settled with azathioprine discontinuation and conservative management. The same patient also failed to respond to golimumab (evidenced by ongoing symptoms and a rise in FC from 467 ug/g at baseline to >1800 ug/g at week 6) and was admitted for rescue infliximab. This too failed and a colectomy was performed, at approximately 8 weeks after his first dose of golimumab. Another patient failed to respond to the first two injections of golimumab and was admitted for deteriorating symptoms at approximately week 3. His oral prednisolone was switched to IV hydrocortisone and golimumab switched to infliximab, to which he responded, and he was discharged. The final SAE (also considered a SAR) involved a patient with active disease despite being established on azathioprine and recent

introduction of prednisolone. Shortly after his second dose of golimumab, he developed lower back pain and fever and was admitted under orthopaedics. An MRI scan showed inflammation in his right L4/L5 facet joint with surrounding fat stranding considered suggestive of infection. In view of this, azathioprine and golimumab were discontinued (his prednisolone reducing regimen had recently completed). He was commenced on IV flucloxacillin and investigations were arranged to exclude tuberculosis. To complete the course of IV antibiotics, at the time of discharge flucloxacillin was switched to ceftriaxone to facilitate daily dosing in an ambulatory care setting.

## Discussion

The GO-LEVEL study includes the largest, prospective, published cohort of patients undergoing detailed PK investigation during induction therapy. In keeping with the results of previous PK studies (Boland et al., 2019; Detrez et al., 2016; Dreesen et al., 2019; Magro et al., 2019b) and post-hoc analysis of the PURSUIT trials (Adedokun et al., 2017), an association between greater drug exposure and favourable treatment outcomes was observed. This included both resolution of symptoms (as judged by indices of clinical disease activity) as well as improvement in objective biochemical markers of disease activity (FC and CRP). Moreover, when these two aspects of tight disease control were combined into a composite endpoint (SCCAI ≤2 *and* FC <250 ug/g), the association remained robust. We observed a median SGC of 5.0 ug/ml in patients who achieved this combined endpoint, compared with 3.0 in those who did not (p=0.02). Further evidence of golimumab's exposure-response relationship was evident in our quartile analyses, which also showed significant trends between exposure, clinical response (p=0.046), clinical remission (p=0.01) and combined clinical-biochemical remission (p=0.01). In addition, greater drug exposure at

week 6 was seen to correlate with reduced clinical disease activity, evaluated using a novel two-item PRO for UC ($r_s$ = -0.36, p=0.03), as well as biochemical disease activity, measured by CRP and FC ($r_s$ = -0.43, p=0.01 and $r_s$ = -0.37, p=0.03, respectively).

The 2017 American Gastroenterological Association guideline on TDM emphasises the need for studies designed to generate data on which therapeutic thresholds for golimumab could be based (Feuerstein et al., 2017). Whilst making recommendations for infliximab, adalimumab, and certolizumab target concentrations, the authors opted not to issue guidance for golimumab due to a lack of sufficient available evidence. At the time of that guideline and the technical review on which it was based (Vande Casteele et al., 2017), the only available golimumab TDM data were from a post-hoc analysis of PURSUIT (Adedokun et al., 2017) and a prospective study of 21 patients (Detrez et al., 2016). These studies both recommended a threshold of approximately 2.5 ug/ml at weeks 6 and 14 to achieve clinical response. Since then, a study investigating endoscopic endpoints has identified a threshold of 7.4,ug/ml for endoscopic remission at week 6 and 3.2,ug/ml at week 14 (Dreesen et al., 2019). As part of our analyses, we identified a week 6 threshold of 3.8 ug/ml as a desirable SGC target for the achievement of both clinical remission and combined clinical-biochemical remission. Taken together and given the various endpoints they target, these thresholds could be considered concordant and appear in keeping with the notion that to achieve 'harder' endpoints requires higher drug levels, i.e., clinical remission requires greater exposure (3.8 ug/ml) than response (2.5ug/ml) and endoscopic remission requires greater exposure still (7.4 ug/ml). This pattern has been adopted previously for the use of older anti-TNF agents (Papamichael et al., 2017; Park et al., 2019).

Although the earliest pharmacokinetic time point studied in GO-LEVEL was week 6, the utility of performing TDM before this has been investigated elsewhere as part of the GO-KINETIC study (Bosca-Watts et al., 2016). In this study of 20 patients, week 2 SGCs did not differ between responders and non-responders at when outcomes were assessed at week 8, regardless of whether this was defined clinically or endoscopically. As SGCs were additionally measured at days 4 and 7, AUC analysis was also carried out to investigate exposure and once again, no difference was found (Bosca-Watts et al., 2016).

We observed an inverse correlation between body weight and week 6 SGC ($r_s$=-0.35, p=0.03) and that subsequently, patients weighing ≥80 kg were significantly less likely to achieve our suggested therapeutic threshold of 3.8 ug/ml (2/11 vs. 16/28, p=0.04). This is likely to reflect the fact that weight-based dosing only takes effect from week 6 onwards. These findings raise the question of whether it would be of benefit for weight-based dosing to commence at treatment initiation (as is the case for infliximab, for example) with patients weighing ≥80 kg receiving higher doses at weeks 0 and 2 as well as from week 6 onwards. Although there is currently no within licence dose-escalation option for patients weighing ≥80 kg, there has recently been a change in golimumab's EMA approval that allows patients weighing <80 kg, who have an inadequate response to induction dosing at weeks 0 and 2, to continue with 100 mg at week 6 and every 4 weeks thereafter, instead of 50 mg. This change took into account the results of a post-hoc analysis of PURSUIT that demonstrated early use of the 100 mg maintenance dose led to achievement of clinical response at week 14 in 28% of patients, who had failed to respond to golimumab at week 6. Early non-responders weighing <80 kg who received the 100 mg maintenance dose were also found to have achieved adequate golimumab concentrations (Philip et al., 2019).

In view of the recent EMA approval change for a specific patient group, our findings along with those of the other PK studies raise a broader question about golimumab dosing; could outcomes be improved by using a higher dose induction regimen, for example, by giving 200 mg doses at weeks 0, 1, 2, 3, and 4 before commencing weight-based maintenance therapy a week 6? This would result in a substantially higher total dose (1000 mg) than the standard regimen (300 mg) or than that studied in phase II of PURSUIT (600 mg), and in addition would closely reflect dosing strategies recently investigated in studies of high dose adalimumab induction for UC and CD in the SERENE trial programme (D'Haens et al., 2019).

Despite the clear association between greater exposure and favourable outcomes at week 6, we did not observe similar associations at weeks 10 or 14, where median SGC in patients achieving combined remission did not differ from those who had ongoing disease activity. In addition, greater exposure at weeks 6 or 10 did not appear to predict outcomes at week 14. This lack of predictive value was also observed in a study by Magro et al. (2019a), who despite observing correlations between week 6 SGC and measures of clinical, biochemical, endoscopic, and histological markers at that time point, did not identify any correlation between week 6 SGC and the same range of measures taken at week 16.

Only one sample was positive for AGA. This was taken at week 6 from a patient who withdrew from the study due to increasing disease activity. Their relevance in relation to his non-response is unclear as they were measured at a low titre (12 ng/ml) and in the presence of low, but not undetectable, drug levels (2.19 ug/ml). However, it is probable that his drug level was suboptimal (at least in part) due to antibody mediated clearance, resulting in inadequate disease control (i.e., PK rather than PD failure). After withdrawing from the study, the patient received rescue infliximab but also failed to respond to this and

subsequently underwent a colectomy. We do not have TDM data to further understand whether this failure was also due to PK reasons (perhaps due to early antibody formation) or whether his disease was genuinely refractory to adequate anti-TNF exposure (i.e., PD failure).

Although not the aim of our study, its prospective design, baseline confirmation of disease activity and serial clinical, biochemical, and QoL evaluations provided the opportunity to evaluate golimumab's efficacy over a range of outcomes. Except for CRP at week 6, we observed significant reductions in clinical (SCCAI and PRO2) and biochemical (FC and CRP) disease activity as well as significant improvement in QoL (IBD-Control-8 and IBD-Control-VAS) at each study time point compared with baseline. Broadly speaking, by week 6 two-thirds of patients achieved a clinical response, half achieved clinical remission, and a third achieved combined clinical-biochemical remission. These rates remained largely stable through to week 14. The efficacy rates observed here appear higher than those seen in PURSUIT-SC where approximate rates of response and remission were 55% and 18%, respectively (Sandborn et al., 2014b). They are also higher than described in some published observational cohorts (Detrez et al., 2016; Magro et al., 2019b), although quite closely concordant with others (Bosca-Watts et al., 2016; Probert et al., 2018). There are several possible explanations for the observed differences but the most likely is that our SCCAI-based definitions are less stringent than those based on Mayo scores. Other possibilities include the relatively high rate of baseline corticosteroid use (44%), the essentially biologic naïve cohort, and the fact that we were able to perform outside of licence dose escalation in the case of predicted or observed suboptimal initial response.

The GO-LEVEL study included the largest published prospective cohort of patients undergoing PK monitoring during induction and early maintenance. Patients were objectively assessed using serial FC measurements and their symptoms were evaluated using both an established disease activity score (SCCAI) and novel PRO (PRO2). These aspects should be considered strengths. However, the study has several limitations. Not least is the lack of endoscopic outcomes, which have become the standard for randomised trials and the widely accepted recommendation for clinical practice (Peyrin-Biroulet et al., 2015). Instead, we used a composite endpoint that included the combination of clinical (SCCAI <3) and biochemical (FC <250) remission to define a pragmatic and clinically relevant treatment outcome. This type of composite endpoint has gained favour for use both in clinical trials (Levesque et al., 2015) as well as clinical practice (Peyrin-Biroulet et al., 2015) (when used as a treatment target). Although current endpoints/targets have relied upon objective assessments being endoscopy-based, efforts have been made to integrate FC into trial outcomes as well as clinical treatment algorithms (Dulai et al., 2019; Pouillon, & Peyrin-Biroulet, 2018). Indeed, many studies have already reported FC-based outcomes (Ma et al., 2018). Investigation has also been carried out into what FC threshold should be used to define remission and although consensus has not been reached, we selected <250 ug/g based on its proven sensitivity and specificity (Lin et al., 2014; Mosli et al., 2015). Although this threshold is debatable, reducing it to 150 ug/g or even 100 ug/g would not have significantly altered our results as only one patient in the combined remission group had an FC >100 ug/g at week 6 and only two at week 14.

Another limitation of our study is the use of a drug-sensitive ADAb assay (LISA TRACKER, Theradiag), meaning that antibodies could only be detected in samples with low or absent

drug levels. Based on previous studies that compared antibody identification rates between drug-sensitive and tolerant assays, this would almost certainly have led to an underestimation of golimumab's immunogenicity. The increase in rates of antibody positive samples was seen to be between 20% and 25% amongst three such studies which ran samples on both types of assay (Adedokun et al., 2017; Adedokun et al., 2019; Detrez et al., 2016). However, the relevance of antibodies found in the presence of adequate drug levels is uncertain. For example, in a study by Detrez et al.(2016) 4/21 (19%) patients were found to have antibodies using a drug-tolerant assay but three of these went on to achieve a partial clinical response, nonetheless. Indeed, there is evidence to suggest that for the use of older anti-TNF agents, antibodies found in the presence of detectable drug levels may not necessarily have a deleterious effect on treatment outcomes (albeit in CD) (Samaan et al., 2016). Finally, our definition of a TL as being within four days of the subsequent administration, rather than immediately before it, could be considered a limitation. This pragmatic definition was used based on logistical factors. Despite the fact that the vast majority of samples were taken on the day of administration, the heterogeneity in sample timing may have had some effect on SGC measurements as the latter are unlikely to remain entirely stable during this sampling window (Detrez et al., 2018).

A fair criticism of observational PK studies, such as our own, is that they can only ever describe an association between drug exposure (however defined) and outcomes. Due to their observational nature, it is impossible to conclusively demonstrate a causal link between inadequate exposure and poor outcomes. It is entirely possible and indeed, mechanistically plausible, that low drug levels are a result of ongoing, refractory disease activity, rather than its cause. For example, receptor-mediated neutralisation due to high

levels of circulating TNF and degradation in an upregulated reticuloendothelial system are two proposed mechanisms by which active inflammation may result in low serum drug levels (Rosen et al., 2015). Another is the loss of therapeutic monoclonal antibodies into stool through the inflamed colonic mucosa, as has been previously described for infliximab (Brandse et al., 2013). However, a recently reported study investigating whether this phenomenon is also a determinant of golimumab's PK showed no evidence that it passed into the stool of UC patients with active inflammation (Berends et al., 2019).

To conclude, there is a clear relationship between golimumab exposure during the initial phase of induction therapy and favourable treatment outcomes including reductions in both clinical and biochemical disease activity. From these findings, it can be inferred that adequate early exposure is required to overcome the inflammatory burden that characterises active UC. Our results suggest that a SGC threshold of 3.8 ug/ml at week 6 most closely associates with achievement of clinical and combined clinical-biochemical remission. Future randomised studies including proactive TDM and serum threshold driven dosing are necessary to further investigate whether these findings represent a causal association.

# Chapter 5: Study of Golimumab Exposure-Response Relationship Using Serum Trough Levels (GO-LEVEL): *Maintenance*

## Aims

The primary aim of the GO-LEVEL maintenance cohort was to define a golimumab trough threshold that is associated with remission during maintenance therapy. The frequency of AGA encountered during the maintenance phase was also investigated, as well as their relation to TL and disease activity.

The data collected as part of GO-LEVEL also allowed validation of a novel UC PRO (PRO2) against an established clinical disease activity score (SCCAI), measures of biochemical disease activity and QoL.

## Methods

### Patients.

As was the case for the GO-LEVEL induction cohort, patients were recruited from the gastroenterology department at GSTT. Patients could be recruited either at the point of flare or during stable remission. Potential study candidates were identified by searching pharmacy records for patients receiving golimumab homecare prescription or at the point of contact with any member of the multidisciplinary GSTT IBD team. Inclusion criteria for the GO-LEVEL maintenance cohort were as follows:

- Aged 18 years or over
- Able to provide written informed consent to participate

- Receiving golimumab treatment for UC for over 14 weeks (having completed six injections at time of screening)
- Sufficient English language skills to understand the patient information sheet and consent form

There were no relevant exclusion criteria and patients who had previously participated in the induction study were permitted to be subsequently recruited to the maintenance cohort.

Demographic information as well as the following disease-related data were collected: disease duration, distribution (using the Montreal classification: Satsangi et al., 2006), BMI, duration and dose of golimumab treatment, prior anti-TNF exposure, concomitant immunomodulation, and corticosteroids.

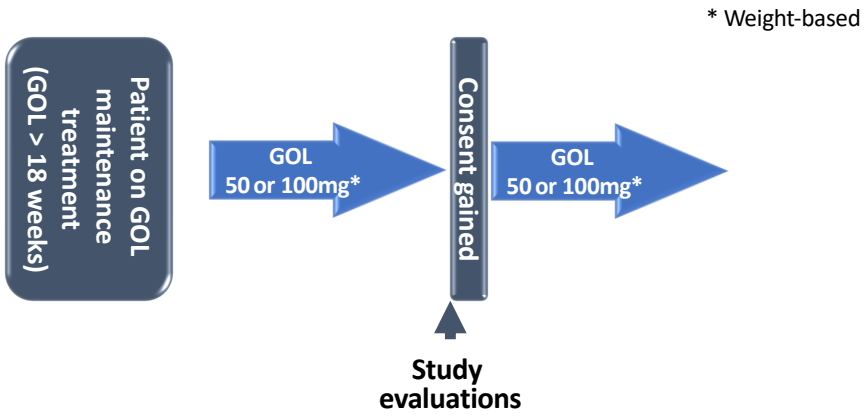
**Golimumab dosing.**

As per the approved dosing strategy, the default maintenance dosing was based on body weight, meaning that patients ≥80 kg received 100 mg, and those <80 kg received 50 mg, every 4 weeks. However, some patients weighing <80 kg had been escalated (outside of licence) from 50 to 100 mg every 4 weeks prior to enrolment. These decisions were made by their supervising physician on clinical grounds and without the benefit of TDM.

**Study evaluations.**

Each study visit included assessments of clinical disease activity, QoL, and biochemical activity. Assessments were arranged to coincide with TL measurements, here defined as within 7 days of the subsequent dose.

Clinical disease activity, QoL and biochemical disease activity were evaluated using the same instruments and definitions as were used for the GO-LEVEL induction cohort. These were SCCAI and PRO2 for clinical disease activity, with clinical remission defined as an SCCAI ≤2. IBD-Control (including a VAS) was used to assess QoL. Biochemical disease activity was measured using FC and CRP, with combined clinical-biochemical remission defined as SCCAI ≤2 *and* FC <250 ug/g. Albumin was also measured as a known determinant of biologic agent PK (see Figure 36 and Table 30).




**Study evaluations**

| | |
|---|---|
| Blood sample: | Golimumab trough level and anti-drug antibodies, CRP |
| Stool sample: | Faecal calprotectin |
| Disease activity indices: | SCCAI, PRO2 |
| Quality of life indices: | IBD-control-8, IBD-Control-VAS |

*Figure 36.* Study evaluations during the GO-LEVEL maintenance study

**Table 30**
*GO-LEVEL maintenance study flow chart*

| | Day 0 | Screen Visit | Day 21-28 | Day 28 |
|---|---|---|---|---|
| Signed Informed consent | | X | | |
| Collection of demographic and UC disease-related data | | X | | |
| Review inclusion/exclusion criteria | | X | | |
| Golimumab administration (self-administered by patients) | X | | | X |
| Serum golimumab concentration and antidrug antibody measurements | | | X | |
| Clinical disease activity scores (SCCAI and PRO2) | | | X | |
| Injection site reaction and IBD-relevant concomitant medication review | | X | | |
| Serum CRP and albumin measurements | | | X | |
| FC | | | X | |
| QoL assessment (IBD-Control) | | | X | |

***Measurement of SGC and AGA concentrations***

Samples for measurement of SGC and AGA concentrations were collected at trough (defined here as within 7 days of the subsequent administration). Samples were processed according to the instructions provided by the manufacturers, using the commercially available LISA TRACKER ELISA (Theradiag, France). This assay is drug-sensitive and is, therefore, only able to detect ADAb when drug levels are low or absent. AGA were considered present at titres ≥10 ng/ml.

### Statistics.

Continuous data are summarised as medians and range (in brackets). Categorical variables were compared using the Fisher's exact or Mann-Whitney U (GraphPad Prism v8.2.1). Correlations between variables were calculated with the Spearman correlation coefficient ($r_s$). ROC curve analysis was used to identify target SGC thresholds. The Chi-squared test for trend (also known as the Cochrane-Armitage test for trend) was used to analyse SGC quartile data. Unless stated, p values are non-significant. All data below/above the limit of quantification were substituted with the value of the lower/upper limit of quantification, i.e., CRP 1 mg/L for levels of <1 mg/L, and FC 4800 µg/g for levels >4800 µg.

### *Ethical & regulatory approval*

This study was granted approval by the MHRA, NHS HRA, and our institute's Gastroenterology Research Project Review Board. It was registered with ClinTrials.gov (NCT03124121) and EudraCT (2017-001374-42).

## Results

### Patient characteristics.

Recruitment commenced in September 2017 and was completed in September 2019. A total of 70 patients receiving golimumab maintenance therapy (defined here as >18 weeks from first dose) were recruited; 67 of these were included in the final analyses. Two patients were excluded from analysis for protocol violations and one patient due to their TDM sample being unsuitable for analysis (Table 31).

**Table 31**
*GO-LEVEL maintenance study patient characteristics*

| Characteristic | n = 67 |
|---|---|
| **Gender, male: female, n (%)** | 37:30 (55:45) |
| **Median age (range), years** | 35.5 (20-73) |
| **Median BMI (range)** | 23.7 (18.2-39.0) |
| **Median disease duration (range), years** | 8 (0.6-28.8) |
| **Median duration on golimumab (range), months** | 6 (5-34) |
| **Disease extent, n (%)** | |
| ***Proctitis*** | 5 (8) |
| ***Left-sided*** | 35 (52) |
| ***Extensive*** | 27 (40) |
| **Concomitant immunomodulation, n (%)** | |
| ***Thiopurine*** | 44 (66) |
| ***Methotrexate*** | 3 (4) |
| **Corticosteroids, n (%)** | 6 (9) |
| **Prior anti-TNF experience, n (%)** | |
| ***Naïve*** | 62 (93) |
| ***Exposed*** | 5 (7) |

**Golimumab dosing.**

Of the 67 patients in the maintenance cohort, 19 weighed 80 kg, or more and 48 were under 80 kg. Of the 48 under 80 kg, 18 (38%) had previously been dose escalated from 50 mg to 100 mg every 4 weeks and 30 (62%) had remained on the standard (licensed) dosing of 50 mg every 4 weeks. The benefit of dose escalation in terms of median SGC was evident

with the 18 dose-escalated patients having significantly higher levels (2.7 ug/ml) than the 30 who remained on standard dosing (2.0 ug/ml, p=0.002). It was also significantly higher than the rest of the cohort as a whole (those weighing <80 kg on 50 mg as well as those weighing ≥80 kg on 100mg), whose median was 2.2 ug/ml (p=0.03). However, when comparing rates of clinical remission in the dose-escalated group (10/18, 56%) with those under 80 kg who remained on standard dosing (20/30, 67%), no significant difference was seen (p=0.54).

A dose proportional relationship was also observed when simply comparing SGC in the patients: 30 patients received 50 mg and 37 100 mg. The median SGC in these groups were 2.0 ug/ml and 3.0 ug/ml, respectively (p=0.0002). However, no correlation was observed between SGC and weight ($r_s$ = -0.07, p=0.6) or BMI ($r_s$ = -0.01, p=0.96).

**Pharmacokinetics.**

The overall median SGC for the 67 patients on maintenance therapy was 2.4 ug/ml (0.6-7.4 ug/ml) and samples were taken at a median of 2 days (0-6 days) prior to the subsequent administration. Of these, 41 (61%) were in clinical remission and 26 (39%) were not. The median SGC of those in clinical remission was 2.6 ug/ml (1.1-6.6 ug/ml) and this did not significantly differ from those who were not at 2.2 ug/ml (0.6-7.4 ug/ml)(p=0.21).

FC data were available for 63 patients and combined clinical-biochemical remission could, therefore, be evaluated. Of these, 31 (49%) were in combined remission and 32 (51%) were not. A significant difference in median SGC was seen when comparing these two groups (2.9 ug/ml vs. 2.1 ug/ml, respectively, p=0.01). Other than SGC, markers of disease activity and QoL measures, no other significant differences were observed between patients who achieved combined remission and those who did not.

The use of concomitant immunosuppressive medication did not appear to have an effect on SGC. The median SGC amongst the 47 patients on immunosuppressants was 2.3 ug/ml compared with 2.6 ug/ml amongst the 20 who were not (p=0.56).

**Table 37**

*Comparison of demographics, clinical characteristics as well as serum golimumab concentration according to clinical remission status during maintenance*

| Characteristic (n=67) | | Clinical remission (n=41) | Not in clinical remission (n=26) | *p*-value |
|---|---|---|---|---|
| **Gender, male: female, n (%)** | | 26:15 (63:37) | 11:15 (42:58) | 0.09 |
| **Median age (range), years** | | 34 (20-73) | 37 (21-68) | 0.80 |
| **Median BMI (range)** | | 23.2 (19.1-39.0) | 25.0 (18.2-35.3) | 0.23 |
| **Median disease duration (range), years** | | 6.7 (0.6-28.8) | 9.5 (1.3-21.4) | 0.40 |
| **Median duration on golimumab (range), months** | | 8 (5-34) | 6 (5-22) | 0.32 |
| **Golimumab maintenance dose, 50 mg:100 mg, n (%)** | | 20:21 (49:51) | 10:16 (38:62) | 0.41 |
| **Concomitant immunomodulator, n (%)** | | 28 (68) | 19 (73) | 0.68 |
| **Prior anti-TNF experience, n (%)** | | 2 (5) | 3 (12) | 0.37 |
| **Corticosteroids, n (%)** | | 2 (5) | 4 (15) | 0.20 |
| **Disease activity** | *Median SCCAI (range)* | 0 (0-2) | 5.5 (3-10) | <0.0001 |
| | *Median PRO2 (range)* | 0 (0-1) | 3 (0-5) | <0.0001 |

| Characteristic (n=67) | | Clinical remission (n=41) | Not in clinical remission (n=26) | *p*-value |
|---|---|---|---|---|
| **Quality of life** | *Median FC (range), ug/g* | 32 (5-1200) | 265 (5-1260) | 0.0057 |
| | *Median CRP (range), mg/L* | 1 (1-7) | 2 (1-21) | 0.010 |
| | *Median albumin (range), g/L* | 47 (40-57) | 46 (40-51) | 0.0031 |
| | *Median IBD-Control-8 (range)* | 16 (7-16) | 5 (0-16) | <0.0001 |
| | *Median IBD-Control-VAS (range)* | 93 (43-100) | 42 (10-81) | <0.0001 |
| **Median serum golimumab concentration (range), ug/ml** | | **2.6 (1.1-6.6)** | **2.2 (0.6-7.4)** | **0.21** |

**Table 38**

*Comparison of demographics, clinical characteristics as well as serum golimumab concentration according to combined clinical- biochemical remission status during maintenance*

| Characteristic (n=63) | | Combined clinical-biochemical remission (n=31) | Not in combined clinical-biochemical remission (n=32) | *p*-value |
|---|---|---|---|---|
| **Gender, male: female, n (%)** | | 21:10 (68:32) | 14:18 (44:56) | 0.06 |
| **Median age (range), years** | | 35 (24-56) | 37 (20-73) | 0.91 |
| **Median BMI (range)** | | 23.2 (19.1-39.0) | 24.2 (18.2-30.9) | 0.63 |
| **Median disease duration (range), years** | | 6.7 (0.6-28.8) | 9.5 (1.3-21.4) | 0.27 |
| **Median duration on golimumab (range), months** | | 8 (4-34) | 5 (4-24) | 0.45 |
| **Golimumab maintenance dose, 50 mg:100 mg, n (%)** | | 13:18 (42:58) | 15:17 (47:53) | 0.79 |
| **Concomitant immunomodulator, n (%)** | | 20 (65) | 26 (79) | 0.32 |
| **Prior anti-TNF experience, n (%)** | | 0 (0) | 3 (9) | 0.24 |
| **Corticosteroids, n (%)** | | 0 (0) | 5 (15) | 0.053 |
| **Disease activity** | *Median SCCAI (range)* | 0 (0-2) | 5 (0-10) | <0.0001 |
| | *Median PRO2 (range)* | 0 (0-1) | 2 (0-5) | <0.0001 |
| | *Median FC (range), ug/g* | 18 (5-207) | 358 (5-1260) | <0.0001 |

| Characteristic (n=63) | | Combined clinical-biochemical remission (n=31) | Not in combined clinical-biochemical remission (n=32) | *p*-value |
|---|---|---|---|---|
| | *Median CRP (range), mg/L* | 1 (1-7) | 1 (1-15) | 0.061 |
| | *Median albumin (range), g/L* | 47 (40-57) | 46 (40-51) | 0.029 |
| **Quality of life** | *Median IBD-Control-8 (range)* | 16 (7-16) | 7 (0-16) | <0.0001 |
| | *Median IBD-Control-VAS (range)* | 93 (45-100) | 51 (10-95) | <0.0001 |
| **Median serum golimumab concentration (range), ug/ml** | | **2.9 (1.1-6.6)** | **2.1 (0.6-7.4)** | **0.01** |

**Quartile analysis.**

To further investigate the exposure-response relationship, quartile analysis was carried out by dividing the cohort into four groups depending on SGC at the time of recruitment (<1.8 ug/ml, 1.8 - <2.4 ug/ml, 2.4 - <3.3 ug/ml, and ≥3.3 ug/ml). A significant trend was observed for combined remission with rates of 31%, 38%, 59%, and 71% for the first, second, third, and fourth quartiles, respectively (p=0.01). However, no significant trend was seen for rates of clinical remission (59%, 47%, 71%, and 69% (p=0.32), respectively).

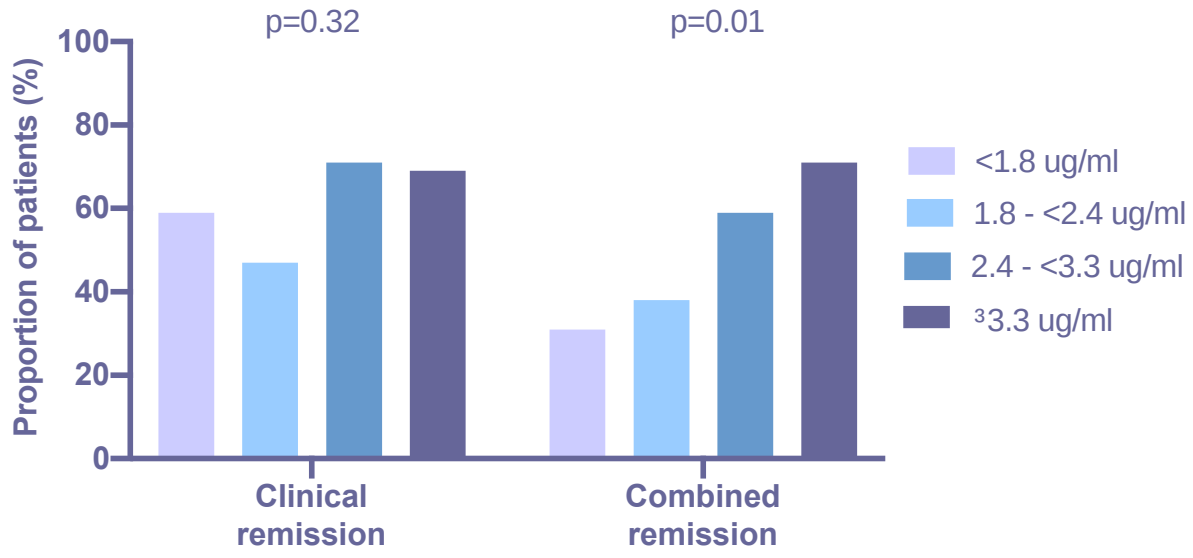



*Figure 37.* Proportion of patients in clinical remission and combined remission according to SGC quartile during maintenance

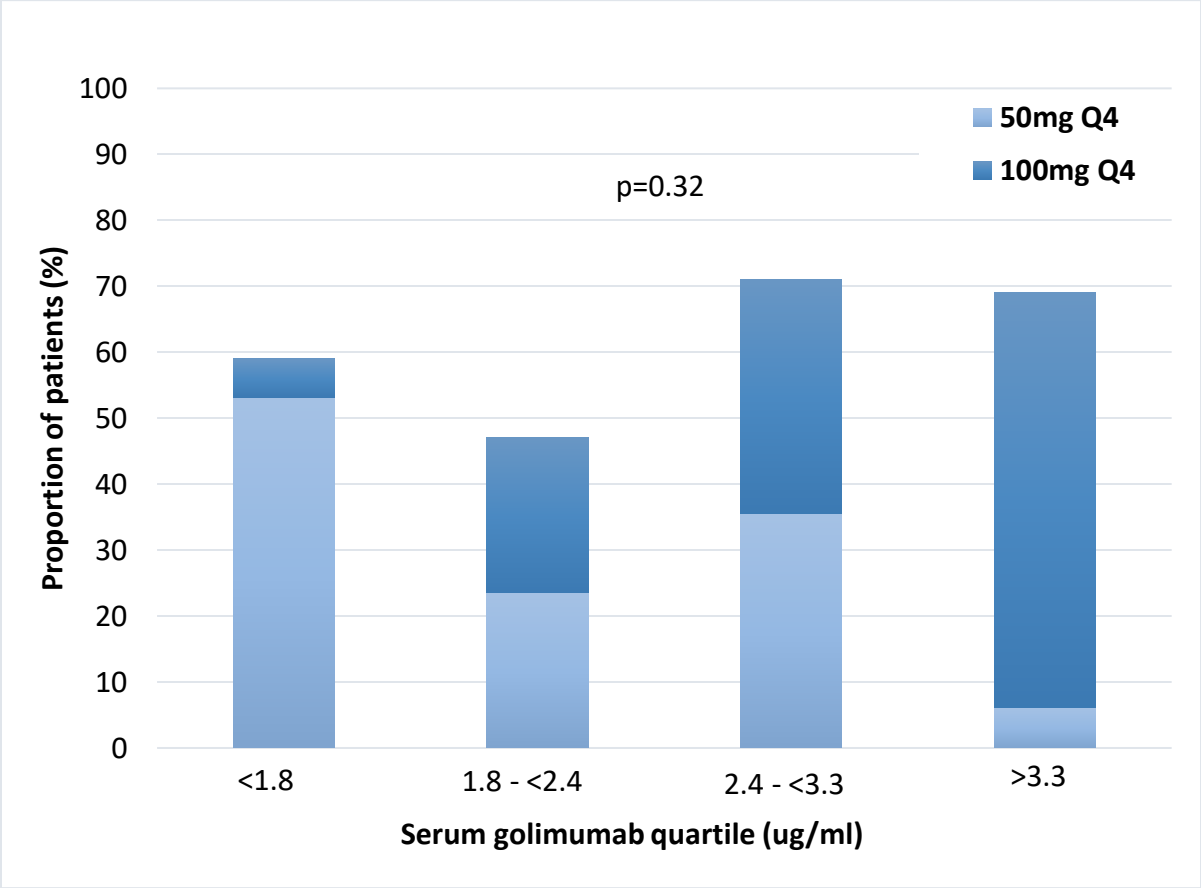



*Figure 38*. Proportion of patients in clinical remission according to SGC quartile during maintenance, divided by maintenance dose

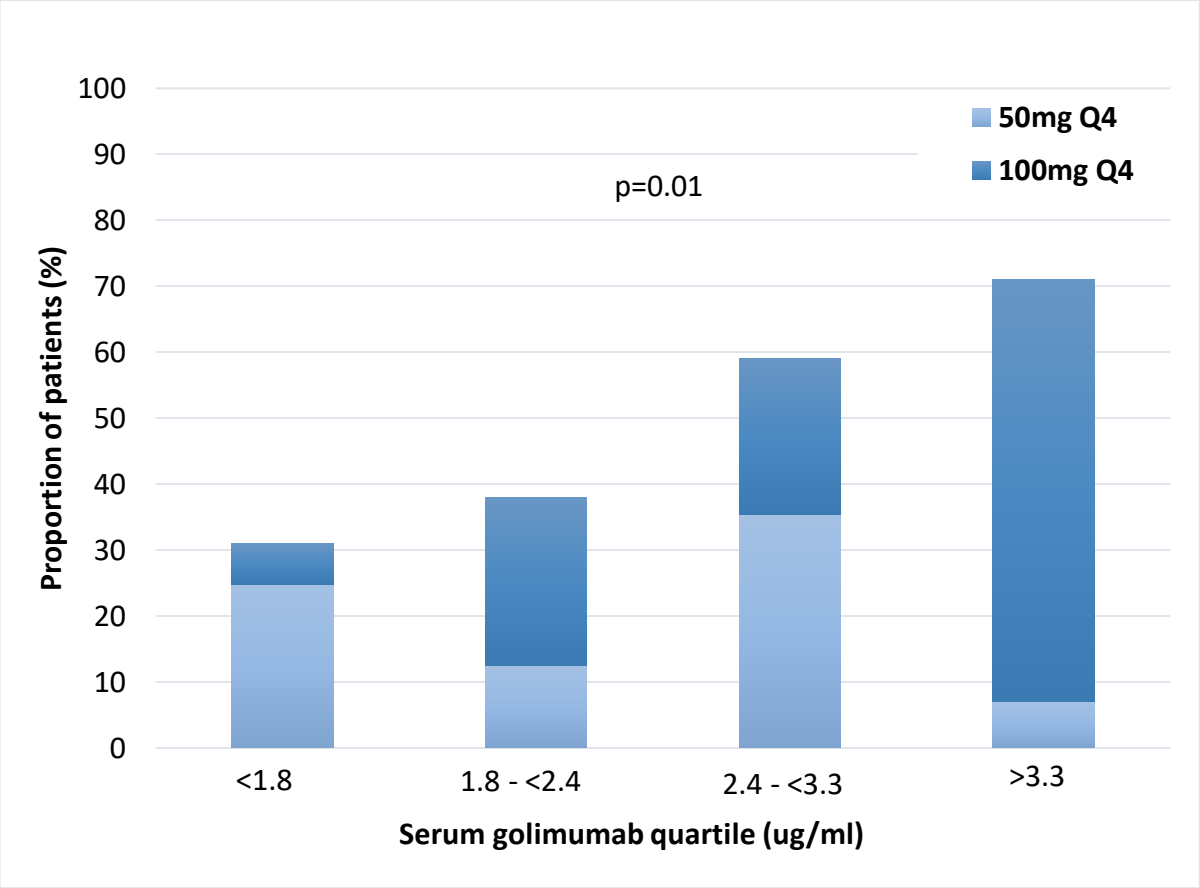



*Figure 39*. Proportion of patients in combined remission according to SGC quartile during maintenance, divided by maintenance dose

**Identification of a target threshold.**

ROC curve analysis was used to identify an SGC threshold that most closely associates with combined clinical-biochemical remission during maintenance. This was found to be 2.4 ug/ml, with an AUROC of 0.68, sensitivity of 0.68, and sensitivity of 0.66 (see Figure 38).

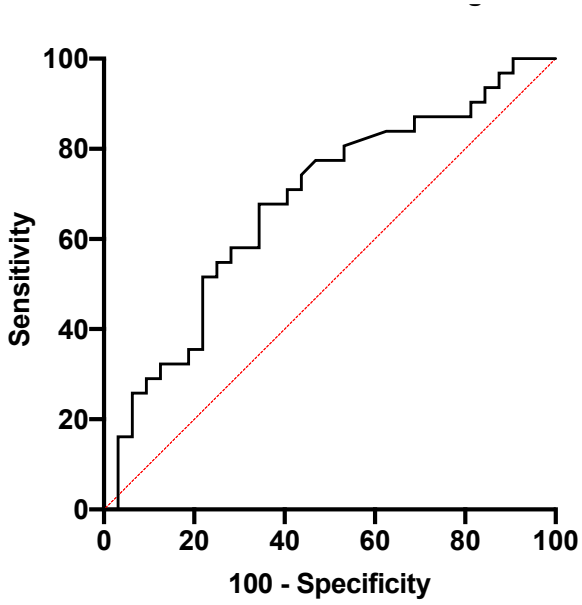



*Figure 38.* ROC curve of optimal SGC associated with combined clinical-biochemical remission during maintenance

**Correlations between SGC, disease activity, and QoL.**

An inverse correlation was observed between maintenance SGC and PRO2 assessments of clinical disease activity ($r_s$ = -0.27, p=0.03). Other than this, no significant correlations were observed (see

Table 39).

**Table 39**

*Correlations between serum golimumab concentrations, disease activity and quality of life*

| Outcomes/biomarkers/demographics | | Maintenance SGC | |
|---|---|---|---|
| | | $r_s$ | *p*-value |
| **Clinical disease activity, median (range)** | **SCCAI** | -0.20 | 0.11 |
| | **PRO2** | -0.27 | **0.03** |
| **QoL, median (range)** | **IBD-Control-8** | 0.18 | 0.14 |
| | **IBD-Control-VAS** | 0.21 | 0.09 |
| **Biochemical disease activity, median (range)** | **CRP** | -0.003 | 0.98 |
| | **FC** | -0.22 | 0.08 |
| | **Albumin** | 0.10 | 0.44 |

**Anti-golimumab antibodies.**

No samples were found to have AGA titres ≥10 ng/ml.

**Validation of PRO2.**

GO-LEVEL recruited a total of 112 patients across the two study cohorts (six patients were subsequently excluded from efficacy and/or PK outcomes). Amongst these, a total of 217 PRO2 assessments were made with concurrent SCCAI and IBD-Control scores available at all time points. CRP measurements were available at 214 of these and FC at 207. Strong correlations were observed between PRO2 and SCCAI ($r_s$=0.94, p<0.0001), as well as with IBD-Control ($r_s$=-

0.82, p<0.0001) and IBD-Control-VAS ($r_s$=-0.78, p<0.0001). Significant correlations were also seen between PRO2 and FC ($r_s$=0.38, p<0.0001) as well as PRO2 and CRP ($r_s$=0.31, p<0.0001) (see Figure 39).

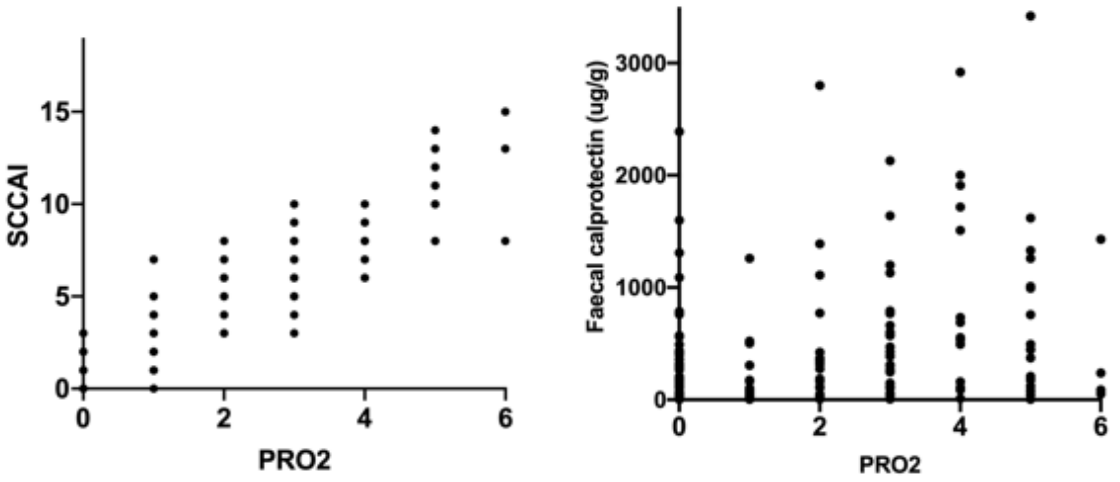



*Figure 39.* PRO2 scores plotted against clinical (SCCAI) and biochemical (faecal calprotectin) disease activity assessments

Of the 217 assessments 109 were in SCCAI defined remission, 95 were in PRO2 defined remission and the two were highly contingent (p<0.0001) (see Table 40). The sensitivity and specificity of disease activity assessments made with PRO2 versus SCCAI were 0.85 and 0.98, respectively. The positive and negative predictive values were 0.98 and 0.87, respectively.

**Table 40**
*PRO2 vs SCCAI defined remission*

| | | SCCAI | |
|---|---|---|---|
| | | **Remission** | **Non-remission** |
| **PRO2** | **Remission** | 93 | 2 |
| | **Non-remission** | 16 | 106 |

**Safety.**

No SAE were reported by patients taking part in the GO-LEVEL maintenance study.

## Discussion

Other than a post-hoc analysis of the PURSUIT trials (Adedokun et al., 2017), our study included the largest published cohort of patients in which the PK profile of golimumab during maintenance therapy was investigated. When using a definition for combined remission that included both clinical and biochemical outcomes (SCCAI ≤2 *and* FC <250ug/g), we observed an exposure-response relationship whereby patients achieving this outcome had a median SGC of 2.9 ug/ml compared to 2.1 ug/ml for those who did not (p=0.01). In keeping with this, SGC quartile analysis showed a significant (p=0.01) trend towards patients with higher levels having an increased likelihood of being in combined remission. In addition, our ROC curve analysis suggested that the optimal SGC threshold to achieve combined remission during maintenance

therapy was 2.4 ug/ml. The findings of our study are consistent with the post-hoc analysis of PURSUIT (Adedokun et al., 2017) as well as a retrospective study by Boland et al. (2019), which both also demonstrated an exposure-response relationship for golimumab during maintenance. Data from the PURSUIT study demonstrated that the optimal SGC threshold to predict clinical remission at week 44 was 1.4 ug/ml (Adedokun et al., 2017) and Boland et al. (2019) suggested a threshold of 5.6 ug/ml for the achievement of mucosal healing, although the number included (n=19) was too small to draw robust conclusions (Boland et al., 2019). Our proposed threshold sits between these two and given the various endpoints they target, they could be considered concordant. The situation here appears similar to the case made earlier regarding targets during induction therapy and the understanding that to achieve 'harder' endpoints requires higher drug levels (Papamichael et al., 2017; Park et al., 2009). In this case, combined clinical-biochemical remission requires greater exposure (2.4 ug/ml) than clinical remission alone (1.4 ug/ml) and endoscopic remission requires greater exposure still (5.6 ug/ml). However, it should be noted that in this cohort and using a purely clinical definition for remission (SCCAI ≤2) we failed to detect a significant difference between groups (median SGC in remission 2.6 ug/ml vs. 2.2 ug/ml in active disease, p=0.21). This is most likely due to the combination of a lack of statistical power due to our modest sample size of 67 (especially compared to the 199 included in the PURSUIT post-hoc maintenance analysis) and limited discriminatory function of the SCCAI, leading to a type 2 error.

In keeping with previous findings, we observed golimumab levels to be dose proportional and as a consequence, that dose-escalating patients from 50 mg to 100 mg improves their PK. However, rates of remission were similar amongst patients who had been dose escalated when

compared with those who remained on standard dosing. There exists conflicting evidence on whether dose escalation is an effective strategy to recapture response in patients who lose response to standard dosing. PURSUIT-M showed that escalation to 100 mg 4-weekly in patients who lost response to initial treatment, did not result in significantly higher response rates compared with patients who continued on 50 mg (34.6% vs. 28.0%, respectively). However, our own previously published observational study as well as a study from KU Leuven both described clinical benefit in small cohorts of dose-escalated patients (Detrez et al., 2016; Samaan et al., 2018b). On the basis that dose escalation has a demonstrable impact on serum levels and that higher levels are associated with clinical and biochemical response, we believe dose escalation from 50 mg to 100 mg 4-weekly still appears a reasonable and rational approach for managing loss of response. As the maximum dose in our cohort was 100 mg 4-weekly, we are unable to comment on the effectiveness of dose escalation beyond but other cohorts (particularly those including CD patients) have described dosing strategies as high as 200 mg every 10 days (Boland et al., 2019; Martineau et al., 2017).

There is currently a great deal of interest in the role of PROs in IBD and their use is rapidly growing. They appear certain to play a role in the future of IBD clinical trials (Williet, Sandborn, & Peyrin-Biroulet, 2014) and are also likely to be integrated into the follow-up of patients during clinical practice (van Deen, Esrailian, & Hommes, 2015). As such, we included a novel two-item PRO for UC (PRO2) as part of our study evaluations (Jairath et al., 2015). PRO2 ranges from 0-6 and consists of the patient derived items from the Mayo score (rectal bleeding and stool frequency). Although PRO2 was internally validated against endoscopic outcomes as part of its development, to the best of our knowledge, it had not yet undergone any process of

external validation. We observed it to perform well when validated against an established clinical disease activity index, QoL assessments, and biochemical markers of disease activity. A PRO2 score of 0 predicted SCCAI-based remission status with accuracy (sensitivity 0.85, specificity 0.98, positive predictive value 0.98, negative predictive value 0.87) and assessments have the benefit of being more rapid to administer than SCCAI, comprising of only two domains compared with six (which includes a total of nine individual items).

We believe the GO-LEVEL maintenance cohort has generated data which could be used to personalise golimumab therapy and maximise its potential benefit. Our proposed threshold could be readily included as part of a treat to trough-target algorithm for golimumab maintenance therapy. Indeed, this is a matter that the American Gastroenterological Association relatively recently identified as requiring further dedicated studies (Feuerstein et al., 2017; Vande Casteele et al., 2017). However, the study has several limitations, most of which are shared with the induction cohort and were discussed previously. These include lack of endoscopic assessments and the use of a drug-sensitive assay. In addition, despite being the largest published cohort to describe the PK of golimumab during maintenance, the study may still have been underpowered to detect a difference between groups based purely on clinical parameters. Finally, our definition of a TL as being within 7 days of the subsequent administration, rather than immediately before it, could also be considered a limitation. Indeed, whether these levels could accurately be described as 'trough' may be a matter for debate. However, this pragmatic definition was chosen based on logistical factors as well as previous data suggesting that TDM for subcutaneously administered anti-TNF (in this case adalimumab) may not necessarily have to be taken just prior to the next scheduled dose (Ward

et al., 2017). TLs in our study were taken a median of 2 days prior to the next scheduled injection and, based on a previous detailed PK study that included repeated samples during a single injection window (Detrez et al., 2018), there is reason to believe this would have had relatively little impact on our findings.

In summary, there exists compelling evidence of an exposure-response relationship for golimumab maintenance therapy. Based on the data presented here, previous studies of golimumab's PK and lessons learnt from older anti-TNF agents, it would appear reasonable to apply TDM to personalise and optimise the use of golimumab. However, to confirm that the observed association between exposure and response is causal will require future, prospective studies, whereby patients are randomised to receive either standard or TDM-based dosing.

# Chapter 6: Conclusions and future work

Based on the results of our own studies, as well as other observational cohorts and large-scale randomised trials, it is clear that golimumab has the potential to deliver significant benefits to patients with UC. These benefits include amelioration of symptoms, such as diarrhoea and rectal bleed, as well as objective markers of inflammation, such as FC and endoscopic features of active disease. In addition, QoL has been observed to improve significantly upon treatment with golimumab. Nonetheless, rates of primary non-response and loss of response remain sizeable and these factors, in addition to a range of practical aspects, limit use of the drug. This limited uptake of use was clearly demonstrated by the UK IBD Registry report published in October 2019, which included 1037 UC patients on biologic therapy, of whom only 59 (6%) were treated with golimumab. This proportion was far lower than those seen for more established anti-TNF agents, with 299 (29%) on adalimumab and 450 (43%) on infliximab (either originator or biosimilar). Since then, the use of golimumab has fallen further behind, likely due to the advent of adalimumab biosimilars and the resulting price-drop as well new advanced therapies, such as tofacitinib and ustekinumab. Indeed, by the time of the IBD Registry's 2021 report, the proportion on golimumab was just 4% (221/5438). Anecdotally, this pattern of use is, at least in part, due to a perceived lack of effectiveness amongst IBD clinicians. Regardless of whether this perception is warranted, it clearly makes sense to define the conditions to derive optimal benefit from the use of golimumab in UC – both from the point of view of the individual patient as well as more broadly in terms of appropriate healthcare resource utilisation.

Our observational study alluded to a link between increasing golimumab exposure (described in that case using the surrogate of dosing, on a mg/kg basis) and improved clinical and biochemical outcomes. The data generated in that study, as well as elsewhere, for empirical dose escalation would serve as reasonable evidence for proof-of-concept in that regard. It is notable that the EMA updated their approved induction dosing to reflect the fact that higher doses than their previous recommendations are likely to be necessary in some patients. They now allow patients with body weight <80 kg who have an inadequate response to induction dosing at weeks 0 and 2, to continue with 100 mg at week 6 and every 4 weeks thereafter, instead of 50 mg. As well as leading to improved clinical outcomes in patients who had failed to respond adequately to induction therapy, a post-hoc analysis of data from PURSUIT-M also showed that this cohort of patients were found to have achieved adequate golimumab levels upon dose escalation (Philip et al., 2019). These types of findings not only have a material benefit in terms of improving patient access to appropriately dosed, effective treatment but also inform future research aimed at further optimisation still.

Our aims for GO-LEVEL were to define therapeutic trough concentration thresholds during induction and maintenance therapy that most closely associate with desirable disease outcomes. This was primarily to inform clinical decision making with regards to dose escalation, such that decisions could be made on the basis of TL (considered in the context of treatment response) rather than on an empirical basis. Concurrently, the study samples were used to verify the use of a commercially available assay for golimumab and AGA concentration measurements. GO-LEVEL generated data that was broadly in keeping with other prospective golimumab TDM studies, as well as post-hoc RCT analyses, and our assay verification

experiments demonstrated adequate operating characteristics of Theradiag's LISA TRACKER. However, as described in an accompanying editorial to GO-LEVEL, written by Roblin, Le Roy, & Paul, (2020), there remain several unanswered questions before golimumab TDM is ready for 'prime time'. Beyond issues regarding inter-assay differences in drug level measurement and drug sensitivity for antibody detection, the most pressing need identified was for interventional studies to demonstrate the utility of TDM-reactive strategies to dose optimise treatment in the case of loss of response. This type of strategy has been shown to be more cost-effective for infliximab use in Crohn's disease when compared to empirical dose escalation (Steenholdt, Brynskov, & Thomsen, 2014; Velayos et al., 2013). It would also have potential benefits in terms of limiting time spent on ultimately ineffective treatments for patients.

Beyond reactive strategies in the setting of loss of response, thoughts could turn to studying the utility of proactive dose optimisation in an attempt to achieve and maintain remission more effectively than standard dosing. This type of approach, however, has yet to be proved effective for other anti-TNF agents, with the TAILORIX study of infliximab in CD showing no significant benefit of using symptoms, biomarkers, and serum drug concentrations to proactively guide dosing (D'Haens et al., 2018).

Ultimately, and even after optimisation, there is likely to be a therapeutic ceiling on what can be achieved with golimumab in UC. As such, attention is switching to combining the various different mechanisms of action now available in an attempt to improve rates of remission. Although combining anti-TNF agents with vedolizumab for patients with IBD and concurrent rheumatological conditions is not entirely uncommon in clinical practice (and a case report

relating to golimumab specifically was published several years ago (Roblin, Paul, & Ben-Horin, 2017), the impact of combining mechanisms solely for IBD-Control remains unclear. A completed but yet to report in full, phase 2a RCT (VEGA) has studied the efficacy and safety of combining golimumab with guselkumab, when compared to either agent used at monotherapy for UC (NCT03662542). Initial data from the induction phase demonstrated that a greater proportion of patients who received combination therapy achieved clinical response at week 12 (83.1%) vs guselkumab (74.6%) or golimumab (61.1%) alone. Similarly, the proportion of patients who achieved clinical remission in the combination group (36.6%) was greater than that of monotherapy groups (21.1% and 22.2%, respectively)(Sands et al., 2022). In the absence of robust biomarkers to predict response to specific therapy, this type of manifold approach, whereby potentially complementary biologic mechanisms are used at appropriate doses to achieve sufficient exposure, may well become the new accepted treatment paradigm for a condition which, despite great strides in recent decades, still has many unanswered questions and significant unmet need.

**Future work.**

There are multiple areas of interest on which future research on the pharmacokinetics and pharmacodynamics of golimumab could focus. One of these is techniques used to measure serum golimumab and anti-golimumab antibodies levels. Whether use of an alternative assay, such as HMSA or RIA, would have generated meaningfully different results is unclear but re-running our samples in this way would certainly be of interest. Similarly, and perhaps more

intriguingly, would be the possibility to re-run our samples using a drug-tolerant antidrug antibody assay. Based on previous data, it is likely that this would yield a higher rate of samples with positive (total) antidrug antibodies. The clinical relevance of this finding remains debatable, but it is probable that this group of patients warrant closer follow-up and repeat TDM sampling to monitor for suboptimal PK and clinical loss of response.

Beyond techniques used for TDM, future work could focus on therapeutic thresholds for use of golimumab in specific settings that are as yet, unexplored. These could include off-label uses such as use in Crohn's disease, where based on its mechanism of action and observational data, golimumab is likely to have some level of efficacy. Along similar lines, investigation of therapeutic thresholds for use in perianal manifestations of Crohn's would also be clinical relevance, as this remains area of significant unmet need and limited therapeutic options. Finally, and perhaps most pertinent to the current direction of travel in IBD medicine, is investigation of therapeutic thresholds when using golimumab in combination with other biologic mechanisms of action (such as, p19 inhibition as is the case with guselkumab). Whether dosing regimens should be altered in any way when used in this setting remains unclear. In addition, the implications of serum concentration and antidrug antibody measurement in the presence of another therapeutic antibody are not yet well understood.

It is entirely possible that these considerations could gain relevance if and when biosimilar versions of golimumab become available, following patent expiry (2024 in EU and US). The corresponding decrease in price may well result in increased use and renewed interest in this range of future work and much more besides.

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# Appendices

**Protocol: Study of the Golimumab Exposure-Response Relationship Using Serum TL (GO-LEVEL)**

# PROTOCOL FULL TITLE: Study of the Golimumab Exposure-Response Relationship using Serum Trough Levels

**Protocol Short Title/Acronym: GO-LEVEL**

**Trial Identifiers**

| | |
|---|---|
| EudraCT Number - | 2017-001374-42 |
| CRN/CPMS - | 34486 |
| IRAS Number - | 194917 |
| ClinTrials.gov - | NCT03124121 |

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**Protocol version 4.1, 13th June 2019**

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## CONTENTS

# 1. Study Synopsis

| | |
|---|---|
| Title of clinical trial | Study of the Golimumab Exposure-Response Relationship using Serum Trough Levels |
| Protocol Short Title/Acronym | GO-LEVEL |
| Trial Phase if not mentioned in title | Phase IV |
| Sponsor name | Guy's & St Thomas' NHS Foundation Trust |
| Chief Investigator | Peter Irving |
| EudraCT number | 2017-001374-42 |
| IRAS number | 194917 |
| Medical condition or disease under investigation | Ulcerative colitis (UC) |
| Purpose of clinical trial | To study the exposure-response relationship of golimumab using serum trough levels |
| Primary objective | To define a week 6 golimumab trough level concentration that predicts response at week 14 |
| Secondary objective (s) | To define golimumab trough level concentrations at weeks 6, 10 and 14 that predict response at each time point during induction therapy, respectively.<br>To define a golimumab trough threshold that is associated with remission during maintenance therapy.<br>Tertiary objectives will centre on the study of the relationship between serum golimumab trough levels and novel disease activity indices (PRO2), biochemical markers |

| | |
|---|---|
| | of disease activity (CRP, faecal calprotectin) and quality of life indices. The role of anti-drug antibodies will also be investigated in relation to trough levels and disease activity. |
| Trial Design | Open-label, non-randomised, phase IV trial.<br>Patients commencing induction therapy with golimumab (cohort 1) will be enrolled into a prospective study.<br>Patients on maintenance golimumab therapy (cohort 2) will be enrolled into a cross-sectional study. |
| Endpoints | Primary: golimumab trough levels and UC disease activity (SCCAI) at weeks 6 and 10<br>Secondary: biochemical markers of UC disease activity (fecal calprotectin and CRP), clinical disease activity (PRO2), development of antibodies and quality of life (IBD-Control) at weeks 6, 10 and 14. |
| Sample Size | Total: 112 patients<br>(cohort 1: 42 patients, cohort 2: 70 patients) |
| Summary of eligibility criteria | Inclusion criteria for cohort 1:<br>• Aged 18 years or over<br>• Written informed consent to participate<br>• Moderate-to-severe UC, defined as:<br>○ SCCAI > 5 *and,*<br>▪ A raised fecal calprotectin (> 59 µg/g) *or,*<br>▪ A raised CRP (> 5 mg/L) *or,*<br>▪ Endoscopic disease activity Mayo 2 or above,<br>***Evaluated within 4 weeks of screening*** |

| | |
|---|---|
| | • Commencing golimumab treatment<br>• Sufficient English language skills to understand the patient information sheet and consent form<br><br>Inclusion criteria for cohort 2:<br><br>• Aged 18 years or over<br>• Written informed consent to participate<br>• Receiving golimumab treatment for UC over 14 weeks (have completed 6 injections at time of screening)<br>• Sufficient English language skills to understand the patient information sheet and consent form<br><br>Exclusion criteria (cohort 1 only)<br><br>• Contra-indication to golimumab: tuberculosis, severe infections or congestive cardiac failure<br>• Imminent need for colectomy (i.e. colectomy is being planned)<br>• Previous primary non-response to anti-TNF therapy in the opinion of the investigator<br>• Previous treatment with more than one anti-TNF therapy (excluding golimumab) |
| IMP, dosage and route of administration | Patients will receive standard induction treatment with subcutaneous golimumab 200 mg at week 0 and 100 mg at week 2. Followed by maintenance treatment of 50 or 100 mg (based on weight) every four weeks until the supervising clinician makes the decision to withdraw treatment (as is the standard of care). |

| | |
|---|---|
| Active comparator product(s) | N/A |
| Maximum duration of treatment of a Subject | Total duration of treatment will be decided by the supervising physician on clinical grounds (exactly as the standard of care) and enrolment into the study will have no bearing on this decision. |
| Version and date of protocol amendments | Version 4.1, 13th June 2019 |

## 2. Glossary of Terms

| | |
|---|---|
| ADA | Anti-drug antibodies |
| CRP | C-reactive protein |
| EMA | European Medicines Agency |
| FDA | US Food and Drug Administration |
| GCP | Good Clinical Practice |
| PK | Pharmacokinetics |
| QoL | Quality of Life |
| RCT | Randomized controlled trial |
| (S)AE | (Serious) Adverse Event |
| Sponsor | The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidizing party. |
| SCCAI | Simple Clinical Colitis Activity Index |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| TNF | Tumor necrosis factor |
| UC | Ulcerative Colitis |

## 3. Background & Rationale

The advent of biologic therapies has led to significant changes in treatment strategies for ulcerative colitis (UC). Prior to biologic therapies, options for treatment primarily consisted of the stepwise use of mesalazine, corticosteroids and immunomodulators for disease of increasing severity. Mesalazine was used to achieve and maintain remission in mild-to-moderate cases with the addition of corticosteroids for those failing to respond or with severe disease. Patients with colitis refractory to intravenous (IV) corticosteroids received ciclosporin or underwent colectomy. Over the past decade, multiple clinical trials have shown the efficacy of anti-TNF therapies for these patients with moderate to severe UC. Therefore, Anti-TNF agents are key tools in current treatment algorithms for both chronically active and acute severe UC.

The effectiveness of biologic agents has also changed treatment goals in ulcerative colitis. This is evident in the evolution of endpoints used for clinical trials and targets used in clinical practice. Conventional and established goals of treatment focused predominantly on achieving symptomatic remission. The cessation of corticosteroid use and achieving mucosal healing were secondary goals. However, in the era of anti-TNF agents with the ability to heal colonic mucosa when other drugs have failed, mucosal healing and steroid-free clinical remission have gained prominence as therapeutic targets.

A significant proportion of UC patients fail to respond to induction therapy with anti-TNF agents (primary non-responders) or require dose escalation due to loss of response over time (secondary non-responders). Dose escalation has been demonstrated to be an effective strategy in patients losing response to anti-TNF therapy. Where this strategy fails or in the presence of significant levels of anti-drug antibodies, switching to another anti-TNF agent (or mechanism of action) is advocated. Therefore, an increase in the range of anti-TNF agents available to clinicians was desired and necessary to overcome the substantial rates of non-response over time. In addition, a better understanding of the effect-response relationship of these agents would allow a more evidence-based approach to dose optimization.

Golimumab represents a new treatment option for patients with moderate-to-severe UC, failing or intolerant of conventional treatments. It is a transgenic, fully human monoclonal immunoglobulin G1 antibody that is synthesized from TNF-immunized transgenic mice expressing human immunoglobulin G. Although it was approved for use in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis in 2009, it was not until 2013 that the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) granted approval for UC.

The PURSUIT (Program of Ulcerative Colitis Research Studies Utilizing an Investigational Treatment) trial program was a series of randomized, double-blind, placebo-controlled studies that led to regulatory approval for the use of golimumab in UC[1,2]. The comprehensive trial program consisted of investigation of the most appropriate route of administration (subcutaneous or intravenous), a phase II dose-ranging study and a phase III trial of induction and maintenance therapy. Subcutaneous administration was found to result in equivalent efficacy and a preferable pharmacokinetic profile when compared with

intravenous dosing and is therefore the approved route of administration. PURSUIT-SC demonstrated that induction therapy with golimumab resulted in a significantly greater proportion of patients achieving a clinical response, clinical remission and mucosal healing at week 6 compared with placebo[1]. All subjects from the PURSUIT-SC study were eligible for enrollment into PURSUIT-M, which evaluated the efficacy and safety of golimumab maintenance therapy over 54 weeks. On-going treatment with golimumab was shown to result in a significantly increased rate of sustained clinical benefit (both response and remission) compared with placebo[2].

However, despite the fact that the PURSUIT trial program yielded positive results and met its primary endpoints, unanswered questions remain regarding the optimal use of golimumab in UC. For example, how could the observed rates of primary and secondary non-response (approximately 50% and 40%) be minimized? In addition to significant rates of non-response, the majority of patients who do respond to the drug remain symptomatic to some degree, are on concomitant steroids, and are without a “normal or inactive” (Mayo 0) mucosal appearance. It’s possible that these outcomes could be improved upon, given a more detailed understanding of the initial exposure-response relationship data that emerged from PURSUIT.

Patients with higher serum concentrations of golimumab were observed to have higher rates of response and remission as well as greater improvement in median composite Mayo scores. In PURSUIT-SC the change from baseline Mayo score and rates of clinical response and clinical remission at week 6 increased with increasing quartiles of serum golimumab concentration. Serum quartile analysis of the subsequent maintenance trial showed that more patients in the higher quartiles achieved clinical response through to week 54, or clinical remission at both weeks 30 and 54, when compared with those in the lower quartiles.

In a recent publication, Adedokun and colleagues reported a rigorous and meticulously performed a study of the pharmacokinetics and pharmacodynamics of golimumab using samples taken as part of the PURSUIT trials. As part of these analyses the authors found serum golimumab concentrations to be dose proportional and that a positive correlation exists between concentrations and efficacy outcomes (clinical response, clinical remission and mucosal healing) during induction and maintenance therapy. They then went further by using receiver-operating-characteristics (ROC) curve analysis to define serum golimumab concentrations that may serve as potential targets for treatment optimization; proposing thresholds of 2.5 µg/ml at week 6 and 1.4 µg/ml during steady-state maintenance therapy[3]. Prior to this, similar findings were also reported by a group from Leuven as part of an observational study of 21 patients being treated with golimumab in a clinical setting. Median golimumab concentrations were significantly higher in partial clinical responders than in non-responders at week 2 (10.0 vs 7.4 µg/ml, $p = 0.035$) and week 6 (5.1 vs 2.1 µg/ml, $p = 0.037$). Their ROC curve analysis revealed a cut-off of 2.6 µg/ml at week 6 (90% specificity, 56% sensitivity, Area Under the Curve 0.79 [95% CI], $p = 0.034$) for the association with a partial clinical response after 14 weeks of treatment[4]. The authors of both of these studies highlighted the need for further prospective trials to validate their findings and add further validation to commercially available assays for the measurement of golimumab serum concentrations.

Data such as these could be used to optimise the use of golimumab in clinical practice and inform prospective therapeutic drug monitoring trials employing trough levels to drive dosing.

Anti-drug antibodies were also detected in a small minority of patients (2.9%) in the PURSUIT trials and the majority of these (67.7%) were found to be neutralizing. Their occurrence was significantly less common in patients who were receiving concomitant immunomodulators (1.1%) compared with patients who were not (3.8%). However, due to the low observed incidence it is difficult to draw conclusions regarding their impact on efficacy. Nonetheless, a clearer understanding of their impact on drug exposure and subsequently, disease activity would be of benefit in defining the optimal use and monitoring of golimumab.

In conclusion, golimumab is a promising new treatment of moderate-to-severe UC. However, several aspects regarding its optimal use remain unclear. Most important of these is the quantification of a minimum exposure threshold that results in a clinical benefit. This requires dedicated clinical trials to generate the necessary evidence to guide clinicians and allow patients to get the most benefit from this new agent.

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# 4 Trial Objectives and Design

## 4.1. Trial Objectives

**Primary Objective**

To define a week 6 golimumab trough level concentration that predicts response at week 14.

**Secondary Objectives**

To define golimumab trough level concentrations at weeks 6, 10 and 14 that predict response at each time point, respectively.

To define a golimumab trough threshold that is associated with remission during maintenance therapy.

**Tertiary Objectives**

Tertiary objectives will centre on the study of the relationship between serum golimumab trough levels and novel disease activity indices (PRO2), biochemical markers of disease activity (CRP, faecal calprotectin) and quality of life indices. The role played by anti-drug antibodies will also be investigated in relation to trough levels and disease activity.

The role played by anti-drug antibodies will be investigated in relation to golimumab trough levels and disease activity.

This study will also generate data that can be used to validate a commercially available golimumab assay as well as a novel patient reported outcome (PRO) assessment of disease activity.

### 4.1.1 Primary endpoints

Drug exposure to golimumab will be evaluated using serum trough level concentrations measured using a commercially available ELISA produced by Theradiag (LISA TRACKER) at weeks 6 and 10. Clinical UC disease activity will be evaluated using SCCAI with the following definitions:

| | |
|---|---|
| Remission | SCCAI ≤ 2 |
| Response | SCCAI ≤ 5, with a decrease by ≥ 2 |
| Relapse | SCCAI ≥ 5 (following a response) |

### 4.1.2 Secondary endpoints

UC disease activity assessments at each time point (weeks 6, 10 and 14) using PRO2, development of anti-drug antibodies, acute infusion reactions (allergic), fecal calprotectin, serum CRP measurements, albumin and QoL assessments using IBD-Control.

## 4.2 Trial Design

This will be an open-label, non-randomised, phase IV trial.

The study will involve two study groups:

Cohort 1 (42 patients): Patients commencing golimumab induction therapy will be included in a prospective, observational study.

Cohort 2 (70 patients): Patients receiving golimumab maintenance therapy will be included in a cross-sectional, observational study.

The study will be initiated and primarily run at Guy's and St Thomas' Hospital, a tertiary IBD referral center. To acquire sufficient patient numbers in a timely manner, patients will also be recruited from Kings College Hospital using pre-existing collaborative research links.

The planned inclusion period is estimated to be one and a half years; by which time the target of 112 patients (between the two study cohorts) will be enrolled.

## 4.3 Trial Flowchart

Patients in Cohort 1 (commencing induction treatment):

| | Screen Visit (day -90 – day 0) | Day 0 | Day 14 | Day 38-42 | Day 42 | Day 66-70 | Day 70 | Day 94-98 | Day 98 |
|---|---|---|---|---|---|---|---|---|---|
| | | Week 0 | Week 2 | | Week 6 | | Week 10 | | Week 14 |
| Signed Informed consent | X | | | | | | | | |
| Collection of demographic and UC disease related data | X | | | | | | | | |
| Review inclusion/exclusion criteria | X | | | | | | | | |
| Golimumab administration (self-administered by patients) | | X | X | | X | | X | | X |
| Serum golimumab concentration and anti-drug antibody measurements | | | | X | | X | | X | |
| Clinical disease activity scores (SCCAI and PRO2) | X | | | X | | X | | X | |
| Injection-site reaction and IBD-relevant concomitant medication review | X[1] | | | X | | X | | X | |
| Serum CRP and albumin measurements | X | | | X | | X | | X | |
| Faecal calprotectin (FC) | X | | | X | | X | | X | |
| Quality of life assessment (IBD-Control) | X | | | X | | X | | X | |

1. Injection site reaction review is not applicable at this visit

Patients in Cohort 2 (on maintenance golimumab treatment):

| | Day 0 | Screen Visit | Day 21-28 | Day 28 |
|---|---|---|---|---|
| Signed Informed consent | | X | | |
| Collection of demographic and UC disease related data | | X | | |
| Review inclusion/exclusion criteria | | X | | |
| Golimumab administration (self-administered by patients) | X | | | X |
| Serum golimumab concentration and anti-drug antibody measurements | | | X | |
| Clinical disease activity scores (SCCAI and PRO2) | | | X | |
| Injection-site reaction and IBD-relevant concomitant medication review | | X | | |
| Serum CRP and albumin measurements | | | X | |
| Faecal calprotectin (FC) | | | X | |
| Quality of life assessment (IBD-Control) | | | X | |

# 5 Trial Medication

## 5.1 Investigational Medicinal Product

Golimumab (Simponi®, Janssen Biotech, Inc., Horsham, PA, USA) is a sub-cutaneously administered anti-TNF agent. A homecare agreement is already in place with the golimumab supplier (MSD) and the medicine will be delivered to the patients' home in the standard manner for each site.

## 5.2 Dosing Regimen

Patients will receive standard golimumab induction treatment of 200 mg at week 0 and 100 mg at week 2, according to standard clinical practice. From week 6 maintenance treatment is started at 100 mg (≥ 80 kg) or 50 mg (< 80 kg) every four weeks. Treatment will be continued until the supervising clinician makes the decision to withdraw treatment (exactly as the standard of care). Enrolment into the trial will have no bearing on this decision.

## 5.3 IMP Risks

In the controlled period of the pivotal trials in rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, and UC, upper respiratory tract infection was the most common adverse drug reaction (ADR) reported in 12.6% of golimumab-treated patients compared with 11.0% of control patients. The most serious ADRs that have been reported for golimumab include serious infections (including sepsis, pneumonia, TB, invasive fungal and opportunistic infections), demyelinating disorders, lymphoma, HBV reactivation, CHF, autoimmune processes (lupus-like syndrome) and haematologic reactions.

## 5.4 Drug Accountability

No accountability as this is part of local site standard care. As patients are being treated as part of standard care, the IMP will be supplied by the NHS (i.e. not by MSD).

## 5.5 Storage of IMP

A homecare plan (to teach patients how to self-administer injections) is already in place with the golimumab supplier (MSD) and the medicine will be delivered to the patients' home in the standard manner. Standard storage information will be given to patients.

## 5.6 IMP labelling

No labelling of the IMP will be required since it is a type A trial and the study drug will be used from commercial stock and according to its SmPC.

## 5.7 Subject Compliance

No specific compliance testing will be carried out but non-compliance could be deduced based on serum drug levels made as part of the trial.

## 5.8 Concomitant Medication

No restrictions on concomitant medications will be made.

Data regarding IBD-relevant concomitant medications ***only*** will be collected at each visit and recorded on the eCRF.

# 6 Selection and Withdrawal of Subjects

## 6.1 Inclusion Criteria

Adult patients with moderate-to-severe UC with an inadequate response to, or unable to tolerate, one or more of the following conventional therapies: oral 5-aminosalicylates, oral corticosteroids, immunomodulators; or are corticosteroid dependent.

Inclusion criteria for cohort 1:

- Aged 18 years or over
- Written informed consent to participate
- Moderate-to-severe UC, defined as:
    - SCCAI > 5 *and,*
        i. A raised fecal calprotectin (> 59 µg/g) *or,*
        ii. A raised CRP (> 5 mg/L) *or,*
        iii. Endoscopic disease activity Mayo 2 or above,

        ***Evaluated within 4 weeks of screening***
- Commencing golimumab treatment
- Sufficient English language skills to understand the patient information sheet and consent form

Inclusion criteria for cohort 2:

- Aged 18 years or over
- Written informed consent to participate

- Receiving golimumab treatment for UC over 14 weeks (have completed 6 injections at time of screening)
- Sufficient English language skills to understand the patient information sheet and consent form

## 6.2 Exclusion Criteria (cohort 1 only)

- Contra-indication to golimumab: tuberculosis, severe infections or congestive cardiac failure
- Imminent need for colectomy (i.e. colectomy is being planned)
- Previous primary non-response to anti-TNF therapy in the opinion of the investigator
- Previous treatment with more than one anti-TNF therapy (excluding golimumab)

There are no relevant exclusion criteria for patients entering cohort 2.

## 6.3 Selection of Participants

At Guy's & St Thomas' Hospital potential participants could be identified by any member of the multidisciplinary direct care team, including registrars, clinical research fellows, consultants as well as clinical nurse specialist and IBD research nurses or pharmacists. Potential participants could be identified during gastroenterology out patient clinics, at endoscopy or during our multidisciplinary meeting ("Virtual Biologics and Immunosuppressant Clinic, VBIC").

**Patients in cohort 1:** The decision to commence golimumab treatment will be made in the patients' best interest along standardised clinical treatment algorithms that are in accordance with NICE guidance. Once this decision has been made potential inclusion in GO-LEVEL will be considered. Patients meeting the inclusion criteria will be invited to take part in the study.

**Patients in cohort 2:** Patients already receiving golimumab treatment will be identified using pharmacy records and patients will be invited to take part in the study.

At King's College Hospital, a similar participant identification plan will be followed with the local Principal Investigator and/or multidisciplinary IBD team identifying potential participants. With the additional step taken that a member of the local clinical care team will contact the potential participant to ask whether they would agree to receive a call from a researcher regarding a study, to which they would be eligible.

To increase recruitment, participants may also be selected using patient identification centres (PIC). Arrangements have been made with Consultant (Dr Leon Pee) and Registrar (Dr Emma Johnston) colleagues at a local secondary care Gastroenterology department: Lewisham University Hospital, Lewisham & Greenwich NHS Trust. A total of approximately 15 minutes per subject is expected to be sufficient for screening records and providing information to potential participants. These additional activities will not be funded but have been agreed with local clinicians, who will also be invited to participate with the publication of the final study results. They may provide a patient information sheet to patients and ask them that if they are interested to contact relevant persons at Guy's & St Thomas'. Alternatively, they may verbally consent patients for their contact details to be forwarded to Guy's & St Thomas'. Once this verbal consent is obtained they will email details using secure @NHS.net to @NHS.net email or by telephone.

## 6.4 Randomisation procedure/Code-break

This is not a randomised study. Patients who are commencing golimumab treatment will be enrolled into cohort 1. Patients who are already on golimumab maintenance therapy will be enrolled into cohort 2. This will be confirmed as an investigator as part of an eligibility review. Every patient will be appointed a sequential two-digit study number.

## 6.5 Withdrawal of Subjects

Participants in both study cohorts have the right to withdraw from the study at any time for any reason. The investigator also has the right to withdraw patients from the study drug in the event of inter-current illness, AEs, SAE's, SUSAR's, protocol violations, cure, administrative reasons or other reasons. It is understood by all concerned that an excessive rate of withdrawals can render the study un-interpretable; therefore, unnecessary withdrawal of patients should be avoided. Should a patient decide to withdraw from the study, all efforts will be made to report the reason for withdrawal as thoroughly as possible.

Should a patient withdraw from study drug ***only***, efforts will be made to continue to obtain follow-up data (including relevant safety assessments), with the permission of the patient. Because this is a non-interventional trial there won't be an interim analysis or premature termination of the study.

Participants who wish to withdraw from trial medication (IMP) will be asked to confirm whether they are still willing to provide the following:

- Trial specific data (clinical and biochemical disease activity scores and quality of life evaluations)
- Data collected as per routine clinical practice

Patient status with regards continuation on the trial will be assessed at every visit and in cases of withdrawal an eCRF withdrawal form will be completed.

### 6.6 Expected Duration of Trial

The end of the trial will be defined as the date of the final database lock. Each individual subject will remain on the trial until they have completed standard induction therapy with golimumab. The visit at week 14 will be the 'end of study visit' and there will be no additional follow-up visits beyond week 14.

## 7 Trial Procedures

## 7.1 By Visit

*Cohort 1*

**Screening visit**

- Signed Informed consent
- Review inclusion/exclusion criteria
- Demographic details: age, gender
- IBD-relevant concomitant medication review (Injection site reaction review is not applicable at this visit)
- Baseline clinical (SCCAI and PRO2) and biochemical assessments (CRP, albumin and FC)
- Baseline quality of life assessment (IBD-Control)
- UC disease related details: anatomic distribution (proctitis, left-sided disease or extensive colitis) and duration of disease

**Day 0 (week 0), day 14 (week 2), day 42 (week 6), day 70 (weeks 10) and day 98 (week 14)**

Patients self-administer golimumab at home.

Any late golimumab administrations (within a week of the planned injection date) would not be considered to significantly impact the integrity of the trial or its results and these will not be considered protocol deviations.

**Day 38-42 (week 6), day 66-70 (week 10), day 94-98 (week 14)**

- Serum golimumab concentration measurement
- Anti-golimumab antibody measurement
- Injection-site reactions and IBD-relevant concomitant medication review
- Clinical (SCCAI and PRO2) and biochemical assessments (CRP, albumin and FC)
- Quality of life assessment (IBD-Control)

In cohort 1 patients commencing induction therapy with golimumab, will receive delivery of the drug and self-injection training from registered nurses under the Homecare agreement already in place. This will be identical to the standard of care provided by the NHS (both at Guy's & St Thomas' and King's College Hospitals). Routine clinical care would usually involve clinical review prior to treatment initiation and again at approximately 10-14 weeks from treatment initiation. GO-LEVEL will include clinical and biochemical assessments made at weeks 6, 10 and 14, and one of these will be arranged to coincide with their routine clinical appointment. Taking part in the study will therefore involve an additional two visits for patients, above routine clinical care. Patients will be asked to self-administer their treatment in the usual way and visits will be arranged such that trough concentrations will be measured within four days ***prior*** to the subsequent dose. Golimumab injections could be given on the same day as the trial visit but assessments and blood tests must be taken ***prior*** to self-administration.

*Cohort 2*

**Screening visit**

- Signed Informed consent
- Review inclusion/exclusion criteria
- Demographic details: age, gender
- Injection-site reaction and IBD-relevant concomitant medication review
- UC disease related details: anatomic distribution (proctitis, left-sided disease or extensive colitis) and duration of disease

**Day 0 (week 0)**

- Patients self-administer golimumab at home

**Day 21-28 (week 4)**

- Serum golimumab concentration measurement
- Anti-golimumab antibody measurement
- Clinical (SCCAI and PRO2) and biochemical assessments (CRP, albumin and FC)
- Quality of life assessment (IBD-Control)

**Day 28**

- Patients self-administer golimumab at home

Any late golimumab administrations (within a week of the planned injection date) would not be considered to significantly impact the integrity of the trial or its results and these will not be considered protocol deviations.

In cohort 2 patients receiving maintenance therapy with golimumab, trough levels will be measured at the next available opportunity after enrollment or at the time of loss of response. In cohort 2, a trough level measurement will be defined as a drug level taken in the final week before the patients next planned injection. Patients may be recruited to cohort 2 in the week leading up to their week 18 injection (i.e. from week 17 after initiation of golimumab onwards).

## 7.2 Laboratory Tests

For both cohorts, at each time point serum golimumab measurements will be made as well as measurements of antibodies to golimumab, serum CRP and albumin. Routine biochemical measurements (CRP and albumin) will be processed in the standard NHS manner. Agreement is in place with our local reference chemistry laboratory (Viapath) for ELISA measurements of golimumab concentrations and anti-drug antibodies using a commercially available assay (LISA TRACKER, produced by Theradiag). Samples from King's College Hospital for golimumab serum concentrations and anti-drug antibody measurement will be transferred to Vipath via an established sample transfer route.

Fecal calprotectin measurements will also be taken at each study time point (i.e. weeks 6, 10 and 14 in cohort 1 and at a single point in cohort 2). The faecal calprotectin samples will be handled in the standard NHS manner, which involves transfer to the reference chemistry laboratory (via an established sample transfer route) at King's College Hospital.

# 8 Assessment of Efficacy

Clinical disease activity will be evaluated at each time point (weeks 6, 10 and 14) using the Simple Clinical Colitis Activity Index (SCCAI) and a recently defined two-item (stool frequency and rectal bleeding) patient reported outcome (PRO2) score. Quality of life will be assessed using the IBD-Control questionnaire.

### 8.1.1 Primary Efficacy Parameters

- Drug exposure to golimumab using serum trough level concentrations.
- Clinical UC disease activity using SCCAI using the following definitions:
  - Remission: SCCAI ≤ 2
  - Response: SCCAI ≤ 5, with a decrease by ≥ 2
  - Relapse: SCCAI ≥ 5 (following a response)

### 8.1.2 Secondary Efficacy Parameters

UC disease activity assessments using PRO2, development of anti-drug antibodies, acute infusion reactions (allergic), fecal calprotectin, serum CRP measurements, albumin, QoL assessments using IBD-Control at each time point.

## 8.2 Procedures for Assessing Efficacy Parameters

For serum trough golimumab levels, anti-drug antibodies and CRP venepuncture will be carried out with 15 ml to be drawn within three days of the subsequent golimumab dose during induction therapy (cohort 1) or one week of subsequent dose during maintenance therapy (cohort 2). Clinical and biochemical disease (calprotectin) activity assessments and quality of life measurements will be collect at weeks 6, 10 and 14 in cohort 1 and week 4 in cohort 2.

# 9 Assessment of Safety

## 9.1 Specification, Timing and Recording of Safety Parameters

General safety assessments will be made as part of each assessment.

## 9.2 Procedures for Recording and Reporting Adverse Events

The Medicines for Human Use (Clinical Trials) Regulations 2004 and Amended Regulations 2006 gives the following definitions:

**Adverse Event (AE):** Any untoward medical occurrence in a subject to whom a medicinal product has been administered including occurrences which are not necessarily caused by or related to that product.

**Adverse Reaction (AR):** Any untoward and unintended response in a subject to an investigational medicinal product which is related to any dose administered to that subject.

**Unexpected Adverse Reaction** (UAR): An adverse reaction the nature and severity of which is not consistent with the information about the medicinal product in question set out in:

The summary of product characteristics (SmPC) for that product (for products with a marketing authorisation)

**Serious adverse Event (SAE), Serious Adverse Reaction (SAR) or Unexpected Serious Adverse Reaction (USAR)**: Any adverse event, adverse reaction or unexpected adverse reaction, respectively, that

Results in death;

Is life-threatening;

Required hospitalisation/prolongation of existing hospitalisation;

Results in persistent or significant disability or incapacity;

Consists of a congenital anomaly or birth defect.

**Important Medical Events (IME) & Pregnancy**

Events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also be considered serious.

Although not a serious adverse event, any unplanned pregnancy will also be reported via the SAE reporting system.

**Reporting Responsibilities**

King's Health Partners Clinical Trials Office (KHP-CTO) is responsible for Pharmacovigilance (as defined in Regulation 5 of the Medicines for Human Use (Clinical Trials) Regulations 2004

All SAEs, SARs and SUSARs will be reported immediately by the Principle Investigator (and certainly no later than 24hrs) to the KHP-CTO in accordance with the current Pharmacovigilance Policy. All SAEs, SARs and SUSARs are to be reported to MSD's Drug Surveillance Department ("MSD DSD") group by the Chief Investigator, including but not limited to all initial and follow up information involving any study subject.

The KHP-CTO will report SUSARs to the regulatory authorities (MHRA, competent authorities of other EEA (European Economic Area) states in which the trial is taking place.

The Chief Investigator will report to the relevant ethics committee. Reporting timelines are as follows:

- SUSARs which are fatal or life-threatening must be reported not later than 7 days after the sponsor is first aware of the reaction. Any additional relevant information must be reported within a further 8 days.
- SUSARs that are not fatal or life-threatening must be reported within 15 days of the sponsor first becoming aware of the reaction.

The Chief Investigator and KHP-CTO will submit a Development Safety Update Report (DSUR) relating to this trial IMP, to the MHRA and REC annually.

### 9.2.1 Adverse events that do not require reporting

AE's will not be collected during the study period but will be managed as per the standard of care. Only injection-site reactions will be collected as AR's during the trial period. They will be managed as per the standard of care.

## 9.3 Treatment Stopping Rules

Because this is a non-interventional trial there won't be an interim analysis or premature termination of the study. The trial may be prematurely discontinued by the sponsor, chief investigator or regulatory authority on the basis of new safety information or for other reasons given by the regulatory authority or ethics committee.

## 10 Statistics

To determine a representative cut-off value for golimumab trough levels between the groups of patients stratified by disease activity/response a receiver-operator characteristics (ROC) curve will be created. A trade-off between sensitivity and specificity will be made to establish an adequate lower margin of the therapeutic golimumab range. This statistical plan was reviewed by an independent statistician at King's College London. Relationships between golimumab trough levels and patient characteristics and clinical parameters (albumin, serum CRP, formation of ADA's, fecal calprotectin and IBD-Control) will be evaluated.

## 10.1 Sample Size

To achieve a power of 80%, with two-sided significance, and to detect a mean difference in serum concentration of 2 mg/L difference a minimum sample size of 42 patients in each cohort would be required. Increasing the sample size beyond this point would achieve greater power to detect smaller differences in serum concentrations between subgroups.

## 10.2 Analysis

Descriptive statistics will be used to analyze baseline characteristics. The primary variable of interest will be golimumab drug levels. Other variables will include: efficacy, gender, age, smoking status, concomitant medication, disease location, disease duration, development of anti-drug antibodies, acute infusion reactions, serum CRP, albumin, fecal calprotectin, SCCAI, PRO2, IBD-Control.

Differences between responders and non-responders will be evaluated using a t-test with the threshold for statistical significance set at 0.05. Univariate and bi-variate analysis will be used to assess factors predicting response, including serum golimumab levels at each time point.

## 11 Direct Access to Source Data and Documents

The Investigator(s) will permit trial-related monitoring, audits, REC review, and regulatory inspections by providing the Sponsor(s), Regulators and REC direct access to source data and other documents (e.g. patients' case sheets, blood test reports, X-ray reports, histology reports etc.).

## 12 Ethics & Regulatory Approvals

The trial will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Research Governance Framework and the Medicines for Human Use (Clinical Trial) Regulations 2004, as amended in 2006 and any subsequent amendments.

This protocol and related documents will be submitted for review to the Research Ethics Committee (REC), and to the Medicines and Healthcare products Regulatory Agency (MHRA) for Clinical Trial Authorisation.

Subsequent protocol amendments will be submitted to the REC and Regulatory Authorities for approval, and that the Chief Investigator will comply with regulations, particularly specifying, Pharmacovigilance reporting and providing the REC & MHRA with progress reports, and a copy of the Final Study Report.

The Chief Investigator will submit a final report at conclusion of the trial to the KHP-CTO (on behalf of the Sponsor), the REC and the MHRA within the timelines defined in the Regulations.

## 13 Quality Assurance

Monitoring of this trial will be to ensure compliance with Good Clinical Practice and scientific integrity will be managed and oversight retained, by the KHP-CTO Quality Team.

## 14 Data Handling

The Chief Investigator will act as custodian for the trial data. The following guidelines will be strictly adhered to:

Patient data will be anonymised

- All trial data will be stored in line with the Medicines for Human Use (Clinical Trials) Amended Regulations 2006 and the Data Protection Act and archived in line with the

Medicines for Human Use (Clinical Trials) Amended Regulations 2006 as defined in the Kings Health Partners Clinical Trials Office Archiving SOP.

## 14.1 Source Data

Source data regarding eligibility review, demographic details, UC disease-related details, injection-site reactions and concomitant medication as well as clinical disease activity (SCCAI and PRO2) will be documented in the patients electronic notes. Data regarding biochemical disease activity will be printed from EPR and stored in a study specific folder along with quality of life questionnaires. Golimumab serum concentrations and anti-drug antibody measurements will also be stored in the study specific folder.

# 15 Data Management

Anonymised patient data will be recorded on a bespoke password protected electronic CRF created by King's Clinical Trials Unit. All patient specific data will be recorded using only this number. The full name and birth date will only be recorded on the informed consent form. The study coordinator will monitor patient inclusion and protocol steps, coordinate data entry, perform data analyses and reporting.

# 16 Publication Policy

Patients are entitled to public disclosure of the results of the trial on the basis of their participation in it. The results of research will be submitted for publication to peer-reviewed scientific journals. Data generated during this study would be of interest and appropriate for publication in the IBD section of high impact general gastroenterology journal (e.g. Gastroenterology, Gut or Clinical Gastroenterology & Hepatology), a specialist IBD journal (Inflammatory Bowel Diseases, The Journal of Crohn's and Colitis) or a specialist Gastroenterology therapeutics journal (Alimentary Pharmacology & Therapeutics). Data in abstract form would be submitted to meetings such as ECCO, BSG, DDW and UEGW.

# 17 Insurance / Indemnity

No trial-specific insurance/indemnity is in place. Standard NHS insurance/indemnity will apply.

## 18 Financial Aspects

Funding to conduct the trial is provided in the form of an initial £81,683 grant from Merck Sharp & Dohme (MSD) with a further £15,547 following the increase in recruitment target.

## 19 Signatures

### 19.1 CI Signature

_____ _____

Chief Investigator Date

Peter M Irving

### 19.2 PI Signature

_____ _____

Principle Investigator Date

## GO-LEVEL Patient Information Leaflet – Cohort 1

**Adult Participant Information Sheet**

**Study of the Golimumab Exposure-Response Relationship using Serum Trough Levels**

*Thank you for taking the time to read this leaflet.*

You are being invited to take part in a research study. Before you decide, it is important that you understand why the research is being carried out and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Do not hesitate to ask us if there is anything that is not clear, or if you would like more information. Please take time to decide whether or not you wish to take part.

**What is the purpose of the study?**

Golimumab is a new and effective treatment for patients suffering from ulcerative colitis (UC). However, some patients treated with golimumab do not experience a significant benefit and even those who benefit initially may find that the effect of the medicine reduces over time.

The purpose of this study is to identify whether these different responses to treatment are linked to levels of golimumab found in the blood of patients treated with the drug. We hope to find a certain blood level of golimumab that will give patients the best chance of having a beneficial effect from the drug. This information will allow golimumab use to be tailored to the individual patient and to be used in a more effective way in the future.

**Why have I been invited to participate?**

You have been asked to participate because you have UC and you are about to start treatment with golimumab.

**Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you decide not to take part it will not affect the standard of care you receive. If you do decide to take part you will be given this information sheet to keep, and be asked to sign a consent form. If you do decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect the standard of care you receive. If you do withdraw from the study, with your consent, we would still like to keep any data collected up until the point of withdrawal.

**What will happen to me if I agree to take part in this study?**

If you agree to take part, you will be offered a series of three appointments with a research doctor over the next three months. Whenever possible these will be scheduled to take place on the day of your out patient appointment and close to your next golimumab dose. If any additional visits become necessary due to being part of the trial, these will be arranged at a time most convenient to you, whenever possible. However, reimbursement will not be offered.

At each of the three visits we will ask you to fill in short questionnaires (about your quality of life and UC symptoms), which will take no more than 20 minutes to complete. All this information will be stored on a secure database and your personal details will not be disclosed to anyone apart from your local gastroenterology team.

At each of the three visits we will ask you for an additional vial of blood (about 15ml - three teaspoons) at the same time as you are having routine blood tests. You will also be asked to bring in a stool sample with you at each of the three visits (collection pots will be sent to you prior to this appointment).

During routine clinical care you would usually be reviewed and asked for blood and stool samples twice during this period, so involvement in the study means one additional visit and sample collection.

If you stop taking golimumab for any reason you will be withdrawn from the study.

**Are there any risks to me?**

You may experience some minor discomfort at the blood testing site but otherwise there are no known risks from taking part in this study. Taking part in the study will not affect your current treatment, nor will it affect your ability to obtain insurance for health purposes.

**Are there any benefits to me?**

The medication you receive will be exactly the same, whether you decide to take part in the trial or not. However, as part of the trial your UC will be more closely monitored in a number of ways, than would be the case in standard care.

**What will happen to my stool samples?**

Your stool sample will be analysed for calprotectin; a marker of gut inflammation and a measure of the activity of your UC. Your sample will be sent to King's College Hospital, where this test is routinely carried out and the results will be sent back to your doctor.

**What will happen to my blood samples?**

Your blood samples will be sent to the laboratory at St Thomas' for analysis. This will include measuring inflammatory markers and golimumab drug levels, as well as tests to determine whether you have produced antibodies to the drug. Antibodies are proteins produced by your immune system to identify and destroy bacteria, viruses and some medicines. With your permission we'd like to store part of your blood sample (serum) for use potential use in future research. All samples will be stored anonymously.

**If I participate will my personal medical information be kept confidential?**

All information that is collected about you during the course of the project will be kept strictly confidential. Any information about you, which leaves the research centre, will have your personal details removed so that you remain anonymous.

Guy's and St Thomas' NHS Foundation Trust (GSTT) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. GSTT will keep identifiable information about you for 5 years after the study has finished, in line with GSTT policy.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained unless you request that it be destroyed. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information
https://www.guysandstthomas.nhs.uk/research/patients/about.aspx

GSTT will use your name and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from GSTT and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The only people in GSTT who will have access to information that identifies you will be people who need to contact you for ongoing research data collection or to audit the data collection process.

**What will happen to the results of the research study?**

We hope to be able to publish the results of this research, and will be happy to provide you with a copy of the publication if you request it. You will not be identifiable in this publication.

**Who is conducting the research?**

This study will be carried out by Dr Mark Samaan (Clinical Research Fellow) under the supervision of Dr Peter Irving (Consultant Gastroenterologist). The study will be run primarily at Guy's and St. Thomas' NHS Trust but will also include patients from other hospitals and the results will form part of Dr Mark Samaan's research degree at King's College London.

**Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people know as the Research Ethics Committee to protect your safety, rights, wellbeing and dignity.

**Who is organising and funding the research?**

This study is funded by an unrestricted educational grant from MSD (a pharmaceutical company).

The sponsor of the research is Guy's and St. Thomas' NHS Trust. The sponsor may decide to stop the study at any time and if this happens the reasons will be explained to you. This will not affect your on-going clinical care. Any anonymised data that has been collected up until this time point will be used for analyses.

**What if there is a problem?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (Dr Peter Irving, peter.irving@gstt.nhs.uk, or Dr Mark Samaan, mark.samaan@gstt.nhs.uk, 02071882499). If you remain unhappy and wish to complain formally, you can do this through the Guy's and St Thomas' Patients Advice and Liaison Service (PALS) on 020 7188 8801, pals@gstt.nhs.uk. The PALS team are based in the main entrance on the ground floor at St Thomas' Hospital and on the ground floor at Guy's Hospital in the Tower Wing.

In the event that something does go wrong and you are harmed during the research and this is due to someone's negligence then you may have grounds for legal action for compensation against Guy's and St Thomas' NHS Trust but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

**I have some further questions, who can I ask?**

If you would like any further details, please contact:

Dr Mark Samaan

Clinical Research Fellow, Guy's & St Thomas' Hospital

Postgraduate Research Student, King's College London

mark.samaan@gstt.nhs.uk

02071882499

Guy's and St Thomas' NHS
NHS Foundation Trust

## GO-LEVEL Patient Information Leaflet – Cohort 2

**Adult Participant Information Sheet**

**Study of the Golimumab Exposure-Response Relationship using Serum Trough Levels**

*Thank you for taking the time to read this leaflet.*

You are being invited to take part in a research study. Before you decide, it is important that you understand why the research is being carried out and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Do not hesitate to ask us if there is anything that is not clear, or if you would like more information. Please take time to decide whether or not you wish to take part.

**What is the purpose of the study?**

Golimumab is a new and effective treatment for patients suffering from ulcerative colitis (UC). However, some patients treated with golimumab do not experience a significant benefit and even those who benefit initially may find that the effect of the medicine reduces over time.

The purpose of this study is to identify whether these different responses to treatment are linked to levels of golimumab found in the blood of patients treated with the drug. We hope to find a certain blood level of golimumab that will give patients the best chance of having a beneficial effect from the drug. This information will allow golimumab use to be tailored to the individual patient and to be used in a more effective way in the future.

**Why have I been invited to participate?**

You have been asked to participate because you have UC and you are about to start treatment with golimumab.

**Do I have to take part?**

No. It is up to you to decide whether or not to take part. If you decide not to take part it will not affect the standard of care you receive. If you do decide to take part you will be given this information sheet to keep, and be asked to sign a consent form. If you do decide to take part, you are still free to withdraw at any time and without giving a reason. This would not affect the standard of care you receive. If you do withdraw from the study, with your consent, we would still like to keep any data collected up until the point of withdrawal.

**What will happen to me if I agree to take part in this study?**

If you agree to take part, you will be offered a series of three appointments with a research doctor over the next three months. Whenever possible these will be scheduled to take place on the day of your out patient appointment and close to your next golimumab dose. If any additional visits become necessary due to being part of the trial, these will be arranged at a time most convenient to you, whenever possible. However, reimbursement will not be offered.

At each of the three visits we will ask you to fill in short questionnaires (about your quality of life and UC symptoms), which will take no more than 20 minutes to complete. All this information will be stored on a secure database and your personal details will not be disclosed to anyone apart from your local gastroenterology team.

At each of the three visits we will ask you for an additional vial of blood (about 15ml - three teaspoons) at the same time as you are having routine blood tests. You will also be asked to bring in a stool sample with you at each of the three visits (collection pots will be sent to you prior to this appointment).

During routine clinical care you would usually be reviewed and asked for blood and stool samples twice during this period, so involvement in the study means one additional visit and sample collection.

If you stop taking golimumab for any reason you will be withdrawn from the study.

**Are there any risks to me?**

You may experience some minor discomfort at the blood testing site but otherwise there are no known risks from taking part in this study. Taking part in the study will not affect your current treatment, nor will it affect your ability to obtain insurance for health purposes.

**Are there any benefits to me?**

The medication you receive will be exactly the same, whether you decide to take part in the trial or not. However, as part of the trial your UC will be more closely monitored in a number of ways, than would be the case in standard care.

**What will happen to my stool samples?**

Your stool sample will be analysed for calprotectin; a marker of gut inflammation and a measure of the activity of your UC. Your sample will be sent to King's College Hospital, where this test is routinely carried out and the results will be sent back to your doctor.

**What will happen to my blood samples?**

Your blood samples will be sent to the laboratory at St Thomas' for analysis. This will include measuring inflammatory markers and golimumab drug levels, as well as tests to determine whether you have produced antibodies to the drug. Antibodies are proteins produced by your immune system to identify and destroy bacteria, viruses and some medicines. With your permission we'd like to store part of your blood sample (serum) for use potential use in future research. All samples will be stored anonymously.

**If I participate will my personal medical information be kept confidential?**

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Guy's and St Thomas' NHS Foundation Trust (GSTT) is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. GSTT will keep identifiable information about you for 5 years after the study has finished, in line with GSTT policy.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained unless you request that it be destroyed. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information
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GSTT will use your name and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from GSTT and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The only people in GSTT who will have access to information that identifies you will be people who need to contact you for ongoing research data collection or to audit the data collection process.

**What will happen to the results of the research study?**

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**Who is conducting the research?**

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**Who has reviewed the study?**

All research in the NHS is looked at by an independent group of people know as the Research Ethics Committee to protect your safety, rights, wellbeing and dignity.

**Who is organising and funding the research?**

This study is funded by an unrestricted educational grant from MSD (a pharmaceutical company).

The sponsor of the research is Guy's and St. Thomas' NHS Trust. The sponsor may decide to stop the study at any time and if this happens the reasons will be explained to you. This will not affect your on-going clinical care. Any anonymised data that has been collected up until this time point will be used for analyses.

**What if there is a problem?**

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (Dr Peter Irving, peter.irving@gstt.nhs.uk, or Dr Mark Samaan, mark.samaan@gstt.nhs.uk, 02071882499). If you remain unhappy and wish to complain formally, you can do this through the Guy's and St Thomas' Patients Advice and Liaison Service (PALS) on 020 7188 8801, pals@gstt.nhs.uk. The PALS team are based in the main entrance on the ground floor at St Thomas' Hospital and on the ground floor at Guy's Hospital in the Tower Wing.

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**I have some further questions, who can I ask?**

If you would like any further details, please contact:

Dr Mark Samaan, Clinical Research Fellow, Guy's & St Thomas' Hospital & Postgraduate Research Student, King's College London

mark.samaan@gstt.nhs.uk, 02071882499

Guy's and St Thomas' NHS
NHS Foundation Trust

# GO-LEVEL Consent form

**Adult Consent Form**

**Study of the Golimumab Exposure-Response Relationship using Serum Trough Levels**

**GO-LEVEL**

Participant Identification Number for this trial:

Researcher:

Please initial box

1. I confirm that I have read the cohort 1/cohort 2 (delete as applicable) information version 2.0 sheet dated July 2017 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from the NHS Trust and Sponsor, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐
4. I understand that the anonymised data generated as part of this study will be made available to researchers in the scientific community, including scientists from the pharmaceutical companies who have supported this study, after its completion. ☐
5. I understand that laboratory results (blood and stool tests) will be made available to my treating team to help with the management of my condition. ☐
6. I agree to part of my blood sample being stored anonymously for use in future research. ☐
7. I agree to take part in the above study. ☐

| Name of participant | Date | Signature |
|---|---|---|
| Name of person taking consent | Date | Signature |

Guy's and St Thomas' NHS NHS Foundation Trust

## GO-LEVEL Study Specific Laboratory Manual

**GO-LEVEL**

**STUDY SPECIFIC LABORATORY MANUAL FOR CARRYING OUT GOLIMUMAB CONCENTRATION AND ANTI-DRUG ANTIBODY ASSAYS**

***SAMPLE COLLECTION AND HANDLING***

1. The tests should be performed on serum or on plasma.
2. Lipaemic sera should be avoided, as well as samples which have been frozen and defrosted more than once.
3. To avoid any non-specific binding, samples which have been frozen for more than 6 months should be centrifuged and filtered.
4. Samples should visually inspected and cloudy samples should not be analysed.
5. Samples should not be kept frozen (-20C) for over 3 years or undergo more than 3 freeze-thaw cycles before analysis

***PROCESSING OF SAMPLES***

1. Where sample storage is necessary, serum samples should be collected in serum separator tubes (SST) and centrifuged at 3000 rpm for ten minutes prior to storage at -20$^{o}$C.
2. Prior to analysis, frozen samples should be thawed on a roller mixer and re-centrifuged.
3. Once thawed, samples should be stored at 2-8$^{o}$C for a maximum of five days prior to analysis.
4. All assays are automated using the DS2 ELISA system (Dynex Technologies)), a fully automated, multi-test and multi-batch immunoassay system.
5. All assays will be performed using the LISA TRACKER (Theradiag, France), enzyme-linked immunosorbent assay (ELISA).

***GOLIMUMAB CONCENTRATION MEASUREMENT***

DS2 automated system is programmed to follow the steps described below:

1. Samples are diluted to 1/101 in dilution and wash buffer (TDL)(e.g. 10µL sample + 1mL TDL) and vortexed vigorously.
2. Diluted samples are added to TNFα coated wells (polystyrene microtiter plate with 6 strips of 8 wells), allowing binding.
3. After 60 minutes incubation, unbound proteins are removed by washing.
4. This wash cycle is repeated a total of three times.
5. Anti-human IgG biotinylated antibodies is added.
6. After 60 minutes incubation, unbound antibodies are removed by washing.
7. Horseradish peroxydase labelled streptavidin is added. The streptavidin binds to the complex formed with biotinylated anti-IgG antibodies.
8. After 30 minutes incubation, the wells are washed again to eliminate any excess of conjugate.
9. The bound enzyme is revealed by addition of substrate TMB (3,3',5,5' tetramethylbenzidin), which after 15 minutes incubation forms a blue colour. The colour intensity is proportional to the amount of Golimumab.
10. Adding sulphuric acid, H2SO4 (0.25M) stops the enzymatic reaction and gives rise to a yellow colouration.
11. After stopping the reaction by adding H2SO4 (0.25M), the optical density is read by a spectrophotometer at 450nm (620 nm reference filter).
12. Calibration standard optical densities will be automatically plotted using a four-parameter logistic (4-PL) curve fit from which golimumab concentrations will be extrapolated and expressed in µg/mL.
13. Samples with results above the measuring range should be re-analysed on dilution with wash buffer (1 in 3 or greater as required).

***ANTI-GOLIMUMAB ANTIBODY CONCENTRATION MEASUREMENT***

1. Samples are diluted to 1/2 in dilution and wash buffer (TDL)(e.g. 130µL sample + 130µL TDL) and vortexed vigorously.
2. Diluted samples are added to golimumab coated wells (polystyrene microtiter plate with 6 strips of 8 wells), allowing binding.
3. After 60 minutes incubation, unbound proteins are removed by washing.
4. This wash cycle is repeated a total of three times.
5. Biotinylated Golimumab is added.
6. After 60 minutes incubation, unbound antibodies are removed by washing.
7. Horseradish peroxydase labelled streptavidin is added. The streptavidin binds to the complex formed with biotinylated Golimumab.
8. After 30 minutes incubation, the wells are washed again to eliminate any excess of conjugate.
9. The bound enzyme is revealed by addition of substrate TMB (3,3',5,5' tetramethylbenzidin), which after 15 minutes incubation forms a blue colour. The colour intensity is proportional to the amount of anti-Golimumab antibodies.
10. Adding H2SO4 (0.25M) stops the enzymatic reaction.
11. After stopping the reaction adding H2SO4 (0.25M), the optical density is read by a spectrophotometer at 450nm.
12. Calibration standard optical densities will be automatically plotted using a four-parameter logistic (4-PL) curve fit from which anti-golimumab antibody concentrations will be extrapolated and expressed in µg/mL.
13. Samples with results above the measuring range should be re-analysed on dilution with wash buffer (1 in 3 or greater as required).

# GO-LEVEL Golimumab sample request form

**Study of the Golimumab Exposure-Response Relationship using Serum Trough Levels (GO-LEVEL)**

Please send samples to: SP Unit, 5th Floor, North Wing, St Thomas' Hospital, Lambeth Palace Road, London, SE1 7EH
Tel: 020 7188 3242

| | | | |
|---|---|---|---|
| Origin of request: (please circle) | Guy's & St Thomas' Hospital | | King's College Hospital |
| R&D number: | 2017-001374-42 | Ethics number: | 194917 |

| Patient Details: Please apply sticker | |
|---|---|
| Subject Number | |
| Name/ Initials | |
| Date of Birth | |

| Tests required: | | |
|---|---|---|
| Tests | **Golimumab serum concentration & Anti-golimumab antibody concentration** | |
| Cohort & time point | **Cohort 1 – time point: Week 6 ☐, Week 10 ☐, Week 14 ☐** | **Cohort 2 (single time point) ☐** |
| Date and time of sample | Signature: | |

Other information:
Collect blood into a serum separation (SST™) or plain tube. One tube is sufficient for both tests.
Centrifuge tube at 3000rpm for 10 minutes, aliquot serum and freeze at -20°C until analysis.
Minimum of 400µL serum required for both tests.

| Client Contact Information: | |
|---|---|
| Point of Contact and direct telephone number | Dr Mark Samaan, 07740637713 |

| For laboratory use only: | | | |
|---|---|---|---|
| Number of samples received: | | | |
| SST/Serum | Sample stored | Database entry completed | |
| | Yes ☐ | Yes ☐ | |

Version 1.0 26th September 2017