

# Profiling Serum and Tissue Biomarkers of Vedolizumab Therapy Response in Inflammatory Bowel Disease.



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# Contents

|                                                                   |           |
|-------------------------------------------------------------------|-----------|
| <b>DECLARATION .....</b>                                          | <b>2</b>  |
| <b>CONTENTS .....</b>                                             | <b>3</b>  |
| <b>THESIS SUMMARY .....</b>                                       | <b>6</b>  |
| <b>ACKNOWLEDGEMENTS.....</b>                                      | <b>9</b>  |
| <b>ABBREVIATIONS .....</b>                                        | <b>10</b> |
| <b>FIGURES.....</b>                                               | <b>14</b> |
| <b>TABLES .....</b>                                               | <b>17</b> |
| <b>CHAPTER 1: INTRODUCTION .....</b>                              | <b>18</b> |
| 1.1 INFLAMMATORY BOWEL DISEASE .....                              | 19        |
| 1.2 EPIDEMIOLOGY .....                                            | 20        |
| 1.3 IMMUNOLOGY OF IBD .....                                       | 22        |
| 1.4 TREATMENT OF IBD .....                                        | 28        |
| 1.4.1 5-amino salicylic acid preparations (5-ASA) .....           | 28        |
| 1.4.2 Glucocorticoids .....                                       | 30        |
| 1.4.3 Thiopurines.....                                            | 31        |
| 1.4.4 Methotrexate .....                                          | 33        |
| 1.4.5 Ciclosporin .....                                           | 34        |
| 1.4.6 Biologic Therapy for IBD .....                              | 36        |
| 1.4.6.1 Anti-Tumour Necrosis Factor Therapies .....               | 36        |
| 1.4.6.1.1 Infliximab .....                                        | 36        |
| 1.4.6.1.2 Adalimumab.....                                         | 38        |
| 1.4.6.1.3 Golimumab .....                                         | 39        |
| 1.4.6.1.4 Etanercept .....                                        | 40        |
| 1.4.6.1.5 Certolizumab Pegol .....                                | 40        |
| 1.4.6.1.6 Comparisons of Anti-TNF Therapies .....                 | 41        |
| 1.4.6.1.7 Biosimilars.....                                        | 41        |
| 1.4.6.1.8 Anti-TNF therapy Summary .....                          | 42        |
| 1.4.6.2 Ustekinumab .....                                         | 42        |
| 1.4.6.3 Risankizumab.....                                         | 43        |
| 1.4.6.4 Combination therapy.....                                  | 44        |
| 1.4.6.5 Drug Monitoring during Biologic Therapy .....             | 46        |
| 1.4.6.6 Dose optimisation strategy.....                           | 46        |
| 1.4.6.7. Novel Oral Agents.....                                   | 51        |
| 1.4.6.7.1 JAK STAT Pathway.....                                   | 51        |
| 1.4.6.7.2 Tofacitinib .....                                       | 52        |
| 1.4.6.7.3 Upadacitinib.....                                       | 54        |
| 1.4.6.7.4 Filgotinib .....                                        | 54        |
| 1.4.6.7.5 Sphingosine-1 phosphate (SIP) receptor modulators ..... | 55        |
| 1.4.6.7.6 Advantages of Novel Oral Agents .....                   | 56        |
| 1.5. BIOMARKERS OF IBD THERAPY OUTCOMES.....                      | 56        |
| 1.5.1 C-Reactive Protein .....                                    | 57        |
| 1.5.2 Serum Albumin .....                                         | 58        |
| 1.5.3 Faecal Calprotectin.....                                    | 58        |
| 1.5.4 Oncostatin M.....                                           | 59        |
| 1.5.5 Tumour Necrosis Factor- $\alpha$ .....                      | 60        |
| 1.6 VEDOLIZUMAB.....                                              | 61        |
| 1.7 THIS THESIS IN CONTEXT .....                                  | 62        |

|                                                                                                                                                     |            |
|-----------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| <b>CHAPTER 2.0: CLINICAL RELEVANCE OF TROUGH DRUG LEVELS OBTAINED DURING MAINTENANCE AND DURING INDUCTION THERAPY WITH VEDOLIZUMAB IN IBD .....</b> | <b>65</b>  |
| 2.1 INTRODUCTION.....                                                                                                                               | 66         |
| 2.2 SPECIFIC AIMS.....                                                                                                                              | 66         |
| 2.3 METHODS .....                                                                                                                                   | 67         |
| 2.3.1 <i>Study Population</i> .....                                                                                                                 | 67         |
| 2.3.2 <i>Ethics and Consent</i> .....                                                                                                               | 68         |
| 2.3.3 <i>Vedolizumab administration</i> .....                                                                                                       | 68         |
| 2.3.3.1 Maintenance study.....                                                                                                                      | 68         |
| 2.3.3.2 Induction study .....                                                                                                                       | 68         |
| 2.3.4 <i>Sample Collection and Analysis</i> .....                                                                                                   | 68         |
| 2.3.4.1 Maintenance study.....                                                                                                                      | 68         |
| 2.3.4.2 Induction study .....                                                                                                                       | 69         |
| 2.3.5 <i>Endpoints and Definitions</i> .....                                                                                                        | 69         |
| 2.3.6 <i>Statistical Analysis</i> .....                                                                                                             | 70         |
| 2.4 RESULTS.....                                                                                                                                    | 70         |
| 2.4.1 <i>Total Patient Population</i> .....                                                                                                         | 70         |
| 2.4.2 <i>Maintenance Study</i> .....                                                                                                                | 72         |
| 2.4.2.1 Maintenance Study Patient Characteristics .....                                                                                             | 72         |
| 2.4.2.2 Maintenance Study: VDZ Trough Concentrations .....                                                                                          | 74         |
| 2.4.2.3 Maintenance study: Association of VDZ Trough Concentrations with Prior or Concomitant Medications .....                                     | 76         |
| 2.4.2.4 Maintenance Study: Association of Trough VDZ Concentration with CRP, Albumin and Calprotectin ..                                            | 78         |
| 2.4.3 <i>Induction Study</i> .....                                                                                                                  | 80         |
| 2.4.3.1 Induction Study Patient Characteristics .....                                                                                               | 80         |
| 2.4.3.2 Induction Study: Treatment Duration .....                                                                                                   | 82         |
| 2.4.3.3 Induction Study: Vedolizumab .....                                                                                                          | 84         |
| 2.4.3.3.1 Vedolizumab: Peak and Trough concentrations.....                                                                                          | 84         |
| 2.4.3.3.1.1 Vedolizumab: Peak and Trough concentrations by Disease Phenotype .....                                                                  | 86         |
| 2.4.3.3.2 Vedolizumab Trough Concentrations and Steroid-Free Remission (SFR) at Weeks 14 and 30.....                                                | 87         |
| 2.4.3.3.2.1 Vedolizumab Trough Concentrations and Steroid-Free Remission (SFR) by Disease Phenotype ..                                              | 89         |
| 2.4.3.3.3 Receiver Operating Characteristics Curve Analyses to Define Vedolizumab Trough Concentration Predictive of Steroid-Free Remission .....   | 90         |
| 2.4.3.3.4 Vedolizumab Trough Concentration and Persistence on Therapy .....                                                                         | 92         |
| 2.4.3.4 Vedolizumab Induction and Biomarkers of Disease Activity: C-Reactive Protein, Serum Albumin, and Faecal Calprotectin .....                  | 93         |
| 2.4.3.4.1 Vedolizumab Induction and CRP .....                                                                                                       | 93         |
| 2.4.3.4.2 CRP and Vedolizumab Trough Concentrations .....                                                                                           | 94         |
| 2.4.3.4.3 Baseline CRP and Week 14 and Week 30 Steroid-Free Remission .....                                                                         | 96         |
| 2.4.3.4.4 Vedolizumab Induction and Serum Albumin .....                                                                                             | 98         |
| 2.4.3.4.5 Vedolizumab Induction and Faecal Calprotectin .....                                                                                       | 101        |
| 2.5 STRENGTHS AND LIMITATIONS .....                                                                                                                 | 101        |
| 2.6 DISCUSSION .....                                                                                                                                | 102        |
| 2.7 CONCLUSION .....                                                                                                                                | 111        |
| 2.8 FUTURE DIRECTIONS .....                                                                                                                         | 112        |
| <b>CHAPTER 3.0: INVESTIGATION OF POTENTIAL SERUM BIOMARKERS OF RESPONSE TO VEDOLIZUMAB IN PATIENTS WITH INFLAMMATORY BOWEL DISEASE.....</b>         | <b>113</b> |
| 3.1: INTRODUCTION.....                                                                                                                              | 114        |
| 3.2: SPECIFIC AIM .....                                                                                                                             | 115        |
| 3.3: METHODS .....                                                                                                                                  | 115        |
| 3.3.1 <i>Ethics and Consent</i> .....                                                                                                               | 115        |
| 3.3.2 <i>Study Population</i> .....                                                                                                                 | 115        |
| 3.3.3 <i>Vedolizumab Administration</i> .....                                                                                                       | 116        |
| 3.3.4 <i>Sample Collection and Analysis</i> .....                                                                                                   | 116        |
| 3.3.5 <i>Endpoints and Definitions</i> .....                                                                                                        | 117        |

|                                                                                                                                                                |            |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------|------------|
| 3.3.6 Biomarker Assays .....                                                                                                                                   | 117        |
| 3.3.7 Statistical Analysis .....                                                                                                                               | 118        |
| 3.4 RESULTS.....                                                                                                                                               | 118        |
| 3.4.1 Patient and Disease Characteristics .....                                                                                                                | 118        |
| 3.4.2 Patient Outcomes.....                                                                                                                                    | 121        |
| 3.4.3 Association of Baseline (Week 0) Concentrations of 54 Inflammatory Markers with Week 14 and Week 30 Steroid Free Remission .....                         | 121        |
| 3.4.4 Baseline Concentrations of Serum Inflammatory Markers and Vedolizumab Treatment Persistence .....                                                        | 128        |
| 3.4.5 Baseline Concentrations of Serum Inflammatory Markers and their Association with Therapeutic Vedolizumab Concentration.....                              | 130        |
| 3.4.6 Concentrations of Serum Inflammatory Markers Immediately Prior to, and at week 6 of Vedolizumab Therapy.....                                             | 132        |
| 3.5 DISCUSSION .....                                                                                                                                           | 136        |
| 3.6 STUDY STRENGTHS AND LIMITATIONS .....                                                                                                                      | 143        |
| 3.7 CONCLUSION.....                                                                                                                                            | 143        |
| <b>CHAPTER 4.0:.....</b>                                                                                                                                       | <b>145</b> |
| <b>PATIENT DERIVED TISSUE EXPLANTS: A POTENTIALLY USEFUL TOOL IN THE INVESTIGATION OF VEDOLIZUMAB AND ITS' IMPACT ON THE GUT TISSUE MICROENVIRONMENT. ....</b> | <b>145</b> |
| 4.1 INTRODUCTION.....                                                                                                                                          | 146        |
| 4.2 SPECIFIC AIMS.....                                                                                                                                         | 149        |
| 4.3. METHODS .....                                                                                                                                             | 149        |
| 4.3.1 Study Population .....                                                                                                                                   | 149        |
| 4.3.2 Ethics and Consent .....                                                                                                                                 | 150        |
| 4.3.3 Endoscopic Examination and Biopsy .....                                                                                                                  | 151        |
| 4.3.4 Preparation of Stock Vedolizumab and Immunoglobulin-G .....                                                                                              | 151        |
| 4.3.5. Ex Vivo Ulcerative Colitis Tissue Explant Culture .....                                                                                                 | 151        |
| 4.3.6 Extraction and Quantification of Biopsy Protein Levels.....                                                                                              | 152        |
| 4.3.7 Determination of Secreted Protein Concentrations in Explant Culture Media .....                                                                          | 153        |
| 4.3.8 Peripheral Blood Mononuclear Cell Preparation .....                                                                                                      | 153        |
| 4.3.9 Peripheral Blood Mononuclear Cell Incubation with Patient Derived Explant Culture Medium ..                                                              | 154        |
| 4.3.10 Assessment of Inflammatory Secretions from PBMC's Following Treatment with Tissue Conditioned Media from UC Explants. ....                              | 155        |
| 4.3.11 Statistical Analysis.....                                                                                                                               | 156        |
| 4.4 RESULTS.....                                                                                                                                               | 158        |
| 4.4.1 Patient and Disease Characteristics .....                                                                                                                | 158        |
| 4.4.2 Association of Inflammatory Protein Secretions in Ulcerative Colitis Patients with and without Prior Anti-TNF Exposure .....                             | 160        |
| 4.4.3 Patient Derived Explant Culture Medium Protein Secretion Following Vedolizumab Incubation                                                                | 163        |
| 4.4.4 The Effect of Vedolizumab Incubation on Eotaxin, Eotaxin-3, and IL-15 Secretion by PBMCs ...                                                             | 167        |
| 4.5 STRENGTHS AND LIMITATIONS .....                                                                                                                            | 169        |
| 4.6 DISCUSSION .....                                                                                                                                           | 170        |
| 4.7 CONCLUSION.....                                                                                                                                            | 177        |
| <b>CHAPTER 5.0: DISCUSSION.....</b>                                                                                                                            | <b>179</b> |
| 5.1: AIMS OF THE THESIS.....                                                                                                                                   | 180        |
| 5.2: SUMMARY OF THESIS FINDINGS.....                                                                                                                           | 180        |
| 5.3: STUDY APPLICATIONS .....                                                                                                                                  | 185        |
| 5.4: OVERALL STRENGTHS AND LIMITATIONS OF THE THESIS.....                                                                                                      | 186        |
| 5.5: AREAS OF FUTURE RESEARCH.....                                                                                                                             | 190        |
| 5.6: CONCLUSIONS.....                                                                                                                                          | 192        |
| <b>REFERENCES .....</b>                                                                                                                                        | <b>193</b> |

# Thesis Summary

Inflammatory bowel disease (IBD) is a chronic progressive disease of the digestive tract characterised by periods of clinical relapses and remission.(1) While biologic therapies are effective, over 50% of patients fail to achieve complete remission and up to 70% require a change of therapy within five years.(2-5) Vedolizumab (VDZ), a humanised monoclonal antibody, specifically recognises the  $\alpha_4\beta_7$  integrin selectively blocking gut lymphocyte trafficking and exerts an anti-inflammatory effect on intestinal tissue.(6, 7) VDZ is effective as induction and maintenance therapy in Crohn's disease (CD)(6) and ulcerative colitis (UC).(7) This thesis evaluates potential predictive markers of VDZ treatment outcome in patients with IBD to enable early identification of responders and non-responders to treatment.

In **Chapter 1**, the history, prevalence, and epidemiology of IBD, the clinical presentations and classifications are recapped. The role of the immune system in IBD pathogenesis, IBD treatment development, and the evidence for current therapies is reviewed. Therapeutic strategies including dual therapy, drug monitoring, and dose optimisation are considered and evidence regarding predictive biomarkers of disease activity and treatment response summarised. The hypotheses are, that there are predictive markers of VDZ treatment outcome and that examination of VDZ trough concentrations and VDZ's impact on inflammatory markers in serum and in the local tissue microenvironment could identify biomarkers predictive of treatment response.

In **Chapter 2**, findings of the clinical utility of two strategies of VDZ drug monitoring; (i) trough levels in patients already established on therapy and (ii) those during induction are presented. Seventy-eight patients were recruited into one of two cohorts; 34 patients established on therapy from whom trough levels were obtained immediately before a routinely scheduled infusion (the maintenance cohort) and 40 patients commencing therapy (the induction cohort). VDZ maintenance trough levels were not significantly associated with disease activity, nor did they correlate with known biomarkers of disease activity (CRP, albumin, and faecal calprotectin). In the induction cohort, with median follow-up of 17.8 months [range 0.5–41 months], complete treatment persistence data was available for 38/40 patients to 20 months and 37 patients to 24 months. Following induction, 18/40 (45%) patients at week 14 and 15/40 patients (40%) at week 30 were in steroid-free remission (SFR). Higher week 6 VDZ trough concentrations were associated with week 14 ( $p=0.015$ ) and week 30 ( $p=0.029$ ) steroid-free remission. Receiver operating curve analysis identified a week 6 trough VDZ of  $\geq 15.5\mu\text{g/mL}$  as predictive of week 14 SFR (sensitivity 82.5%, specificity 65%,  $p=0.017$ ) and week 30 SFR; (sensitivity 86.7%, specificity 63.6%,  $p=0.006$ ).

Those with a week 6 trough concentration of  $\geq 15.5\mu\text{g/mL}$  were also more likely to persist on treatment compared with those with a trough level  $< 15.5\mu\text{g/mL}$  ( $p=0.004$ ).

In **Chapter 3**, whether baseline serum concentrations of 54 inflammatory markers (cytokines, chemokines and growth factors) were predictive of response to VDZ at weeks 14 and 30 was determined. Thirty-nine of 43 recruited patients due to start VDZ were included in the analysis. An enzyme-linked immunosorbent assay (ELISA) kit was used to measure 54 serum analytes at baseline and week 6. In univariate analysis, higher baseline concentration of TNF- $\beta$  ( $p=0.0003$ ) and lower baseline concentrations of IL-22, VEGF-C and IL-7 ( $p=0.034$ ,  $p=0.042$ ,  $p=0.045$ , respectively) were associated with week 14 steroid-free remission. Higher baseline concentrations of IL-4, MDC, and MCP-4 ( $p=0.011$ ,  $p=0.026$ ,  $p=0.028$  respectively) and lower concentrations of IL-10 ( $p=0.03$ ) were associated with week 30 steroid-free remission. All markers fell short of the threshold for significance following multiple test correction. At week 6, following VDZ induction, after correction for multiplicity, serum eotaxin and eotaxin-3 concentrations were significantly higher ( $p=0.0012$ ,  $p=0.0013$ , respectively) and serum IL-15 significantly lower compared to baseline ( $p=0.0004$ ), however these changes did not correlate with clinical status or response to treatment.

In **Chapter 4**, in two separate patient cohorts, the UC explant model was used to determine the effect of prior anti-TNF therapy on the colonic secretome, i.e., the collection of molecules and biologic factors excreted by cells into the extra cellular space, and VDZ's impact on the secretome as a reflection of *in-vivo* changes in the gut microenvironment. Using the UC explant model, cross-talk between the UC explant secretome and peripheral blood mononuclear cells was examined.

Prior anti-TNF therapy had no effect on the 35 inflammatory markers analysed in the UC explant secretome. Onset of VDZ action on the UC explant secretome was rapid with alterations detected following a 24-hour VDZ incubation. Concentrations of IP-10, MDC and IL-21 were lower ( $p=0.016$ ,  $p=0.039$ ,  $p=0.05$ , respectively) in VDZ treated samples compared to controls. Lower concentrations of IL-15 were found in the supernatant of PBMCs stimulated with VDZ treated explant culture medium compared to controls. Notably IL-15 serum concentrations were also decreased following VDZ induction (**Chapter 3**).

In **conclusion**, this thesis responds to the challenge to identify VDZ responders for whom durable remission might be anticipated and those at risk of treatment failure. Week 6 was identified as an important time point during therapy. Week 6 trough levels can be predictive of treatment outcome. Baseline CRP can identify those at risk of failure and baseline CRP  $< 5\text{mg/L}$  was associated with higher VDZ trough levels. The UC explant model revealed the rapidity of VDZ action on the colonic secretome which could be independent of the  $\alpha_4\beta_7$  integrin pathway. While

no single biomarker of outcome was identified, several potential leads for further investigation were found. The findings add to the existing knowledge base and pave the way toward identifying a biomarker predictive of treatment response that could enable a more individualised patient-tailored approach to treatment selection.

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# Abbreviations

|         |                                                                                                       |
|---------|-------------------------------------------------------------------------------------------------------|
| 5-ASA   | 5-Aminosalicylic acid                                                                                 |
| ACCENT  | A CD Clinical trial evaluating Infliximab (IFX) in a new Long-term Treatment Regimen                  |
| ADA     | Adalimumab                                                                                            |
| AMPS    | Antimicrobial peptides                                                                                |
| APCs    | Antigen presenting cells                                                                              |
| AUC     | Area under the curve                                                                                  |
| AZA     | Azathioprine                                                                                          |
| BCA     | Bicinchoninic acid                                                                                    |
| BSA     | Bovine serum albumin                                                                                  |
| CD      | Crohn's disease                                                                                       |
| CDAI    | Crohn's Disease Activity Index                                                                        |
| CLASSIC | Clinical Assessment of Adalimumab Safety and Efficacy Studied as Induction Therapy in Crohn's Disease |
| CRP     | C-reactive protein                                                                                    |
| EDTA    | EthyleneDiamineTetraAcetic acid                                                                       |
| ELISA   | Enzyme-Linked Immunosorbent Assay                                                                     |

|               |                                                                        |
|---------------|------------------------------------------------------------------------|
| FBC           | Full Blood Count                                                       |
| FBS           | Foetal Bovine Serum                                                    |
| FDR           | False discovery rate                                                   |
| GALT          | Gut Associated Lymphatic Tissue                                        |
| GI            | GastroIntestinal                                                       |
| HBI           | Harvey Bradshaw Index                                                  |
| Hz            | Hertz                                                                  |
| IBD           | Inflammatory Bowel Disease                                             |
| IBD-U         | Inflammatory Bowel Disease-unclassified                                |
| IFN- $\gamma$ | Interferon-gamma                                                       |
| IFX           | Infliximab                                                             |
| IgG           | Immunoglobulin                                                         |
| IL            | Interleukin                                                            |
| INITIative    | Investigator Network for Inflammatory bowel disease Therapy in Ireland |
| IP-10         | Interferon- $\gamma$ -inducible protein                                |
| IQR           | interquartile range                                                    |
| IV            | Intravenous                                                            |

|          |                                                                 |
|----------|-----------------------------------------------------------------|
| JAK-STAT | Janus kinase-signal transducers and activators of transcription |
| MAdCAM-1 | Mucosal vascular Addressin-Cell Adhesion Molecule-1             |
| MDC      | Macrophage Derived Chemokine                                    |
| mg       | Milligram                                                       |
| mL       | Millilitre                                                      |
| MTX      | Methotrexate                                                    |
| nm       | Nanometer                                                       |
| NOD      | Nucleotide-binding Oligomerisation Domain                       |
| OR       | Odds Ratio                                                      |
| PAMP     | Pathogen Associated Molecular Patterns                          |
| PBMC     | Peripheral Blood Mononuclear Cell                               |
| PBS      | Phosphate buffered saline                                       |
| pg/mL    | Picograms per millilitre                                        |
| PRRs     | Pattern Recognition Receptors                                   |
| RIPA     | Radioimmunoprecipitation Assay buffer                           |
| RNA      | Ribonucleic acid                                                |
| ROC      | Receiver Operating Characteristics                              |

|      |                               |
|------|-------------------------------|
| RPM  | Revolutions Per Minute        |
| RTP  | Room Temperature and Pressure |
| SFR  | Steroid Free Remission        |
| TLRs | Toll Like Receptors           |
| TNF  | Tumour Necrosis Factor        |
| Treg | T regulator lymphocytes       |
| UC   | Ulcerative Colitis            |
| VDZ  | Vedolizumab                   |
| μL   | Microlitre                    |

# Figures

|                   |                                                                                                                                                                                                                                           |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 1.1        | Clinical and endoscopic features of IBD                                                                                                                                                                                                   |
| Figure 1.2        | Age-standardised prevalence rate of IBD per 100,000 population                                                                                                                                                                            |
| Figure 1.3        | Defence mechanisms of the healthy intestinal mucosal barrier                                                                                                                                                                              |
| Figure 2.1        | Vedolizumab trough concentration study flow diagram                                                                                                                                                                                       |
| Figure 2.2        | Boxplots showing the median, IQR and range of vedolizumab trough levels ( $\mu\text{g/mL}$ ) of 34 patients with IBD on maintenance therapy                                                                                               |
| Figure 2.3        | Boxplots showing the median, IQR and range of vedolizumab trough levels ( $\mu\text{g/mL}$ ) during maintenance therapy with and without other prior or concomitant IBD therapies                                                         |
| Figure 2.4        | Scatterplots showing the relationship between vedolizumab trough concentration ( $\mu\text{g/mL}$ ) during maintenance therapy and concomitant serum CRP ( $\text{mg/L}$ ) and albumin ( $\text{g/L}$ )                                   |
| Figure 2.5        | Vedolizumab persistence in the induction study cohort                                                                                                                                                                                     |
| Figure 2.6        | Vedolizumab persistence in anti-TNF Naïve and anti-TNF treated patients                                                                                                                                                                   |
| Figure 2.7        | Boxplots showing the median, IQR and range of vedolizumab serum concentrations ( $\mu\text{g/mL}$ ) during induction therapy                                                                                                              |
| <b>Figure 2.8</b> | Boxplots showing the median, IQR and range of vedolizumab serum concentrations ( $\mu\text{g/mL}$ ) for patients with CD and UC during induction therapy                                                                                  |
| Figure 2.9        | Boxplots Showing the median, IQR, and range of vedolizumab trough concentrations ( $\mu\text{g/mL}$ ) at weeks 2 and 6 in those with and without steroid free remission at weeks 14 and 30                                                |
| Figure 2.10       | Receiver operating characteristic (ROC) curve of vedolizumab trough levels as predictors steroid free remission                                                                                                                           |
| Figure 2.11       | Kaplan-Meier survival curves showing survival free of vedolizumab discontinuation in those with week 6 vedolizumab trough concentrations $\geq 15.5\mu\text{g/mL}$ compared with those with a trough concentration $< 15.5\mu\text{g/mL}$ |

|             |                                                                                                                                                                                                        |
|-------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Figure 2.12 | CRP levels during vedolizumab induction                                                                                                                                                                |
| Figure 2.13 | Boxplot showing the median, IQR and range of week 6 vedolizumab trough levels ( $\mu\text{g/mL}$ ) in patients with a baseline CRP <5mg/L and $\geq 5\text{mg/L}$                                      |
| Figure 2.14 | Scatterplots showing the relationship between vedolizumab trough levels ( $\mu\text{g/mL}$ ) and the concomitant CRP (mg/L) at the second, third and fourth vedolizumab infusion                       |
| Figure 2.15 | Boxplots showing the median, IQR and range of baseline CRP (mg/L) in those with and without steroid free remission at week 14 and week 30                                                              |
| Figure 2.16 | Boxplots showing the median, IQR and range of week 6 vedolizumab trough concentrations ( $\mu\text{g/mL}$ ) in those with baseline serum albumin <40g/L and $\geq 40\text{g/L}$                        |
| Figure 2.17 | Scatterplots showing the relationship between vedolizumab trough concentration ( $\mu\text{g/mL}$ ) and the concomitant serum albumin (g/L) prior to the second, third and fourth vedolizumab infusion |
| Figure 2.18 | Boxplots showing the median, IQR and range of baseline serum albumin (g/L) in those with and without steroid free remission at week 14 and week 30                                                     |
| Figure 3.1  | Serum Biomarker Study Flow Diagram                                                                                                                                                                     |
| Figure 3.2  | Baseline concentrations of TNF- $\beta$ , IL-22, VEGF-C and IL-7 in those with and without week 14 steroid free remission                                                                              |
| Figure 3.3  | Baseline concentration of IL-4, MDC, IL-10 and MCP-4 in those with and without week 30 steroid-free remission                                                                                          |
| Figure 3.4  | Persistence on vedolizumab therapy by inflammatory marker concentration                                                                                                                                |
| Figure 3.5  | Baseline IL-7 concentrations for those with vedolizumab concentration $\leq 12\mu\text{g/mL}$ and $>12\mu\text{g/mL}$ following induction                                                              |
| Figure 3.6  | Eotaxin, Eotaxin-3 and IL-15 concentrations at baseline (week 0) and at week 6                                                                                                                         |
| Figure 4.1  | UC Explant Study Flow Diagram                                                                                                                                                                          |
| Figure 4.2  | IP-10, MDC, and IL-21 concentrations in conditioned media of VDZ treated UC explants compared with IgG treated controls                                                                                |

Figure 4.3 IL-15 concentration PBMCs supernatant following incubation with either VDZ treated or IgG treated UC explant conditioned media

# Tables

|            |                                                                                                                                                              |
|------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Table 2.1  | Demographics and clinical characteristics of maintenance study participants at enrolment a                                                                   |
| Table 2.2. | Demographics and clinical characteristics of induction study participants at time of vedolizumab initiation (n=40)                                           |
| Table 2.3  | Vedolizumab trough concentrations (week 2 and week 6) in patients with and without steroid free remission at weeks 14 and 30 stratified by disease phenotype |
| Table 3.1  | Demographics and clinical characteristics of 39 patients with inflammatory bowel disease at time of vedolizumab initiation                                   |
| Table 3.2  | Baseline concentrations, median [IQR], of 54 inflammatory markers by disease status i.e., persistent disease or steroid free remission at 14 and at 30 weeks |
| Table 3.3  | Concentrations (pg/mL) of 54 inflammatory markers, median [IQR], at baseline and at week 6 of vedolizumab induction.                                         |
| Table 4.1  | Demographics and clinical characteristics of 24 patients with ulcerative colitis                                                                             |
| Table 4.2  | Concentrations, median [IQR], of 35 inflammatory proteins in UC explant conditioned media from patients with and without prior anti-TNF therapy              |
| Table 4.3. | Concentrations, median [IQR], of 54 inflammatory proteins in UC explant conditioned media following treatment with vedolizumab or with IgG (control)         |

# Chapter 1: Introduction

## 1.1 Inflammatory Bowel Disease

Inflammatory bowel disease (IBD) is a chronic progressive disease of the digestive tract characterised by periods of clinical relapse and remission. It can present with weight loss, chronic diarrhoea (often bloody), abdominal pain, and failure to thrive.(1) Three categories are recognised; Crohn's disease (CD), Ulcerative colitis (UC) and Inflammatory Bowel Disease-unclassified (IBD-U). Crohn's disease (CD) is a transmural inflammatory condition of the gastrointestinal (GI) tract that can affect any area of the alimentary canal, often in a discontinuous fashion. Penetrating complications such as abscesses and fistulae and development of fibrotic luminal strictures are not uncommon.(8) UC is characterised by an inflammatory process that primarily affects the colonic mucosa with continuous inflammation proximal to the anal canal. It does not affect the small bowel.(9) Prior to Mummery's description of colonic CD in 1960, the prevailing belief was that CD could not affect the colon. In his seminal paper, Mummery described 25 cases of CD involving the large intestine, and highlighted the clinical, radiological, and pathologic features that differentiated colonic CD from UC.(10)

IBD-U, previously referred to as indeterminate colitis, was first described in 1978.(11) It accounts for the 5–15% of IBD cases that have an overlapping clinical phenotype such that one cannot reliably distinguish between CD and UC.(12)

There is no single reference standard for the diagnosis of IBD; either CD, UC, or IBD-U, rather the diagnosis is based on a combination of clinical, biochemical, endoscopic, and histological findings in a setting where other aetiologies such as infective, ischaemic and drug induced causes have been excluded.(13)

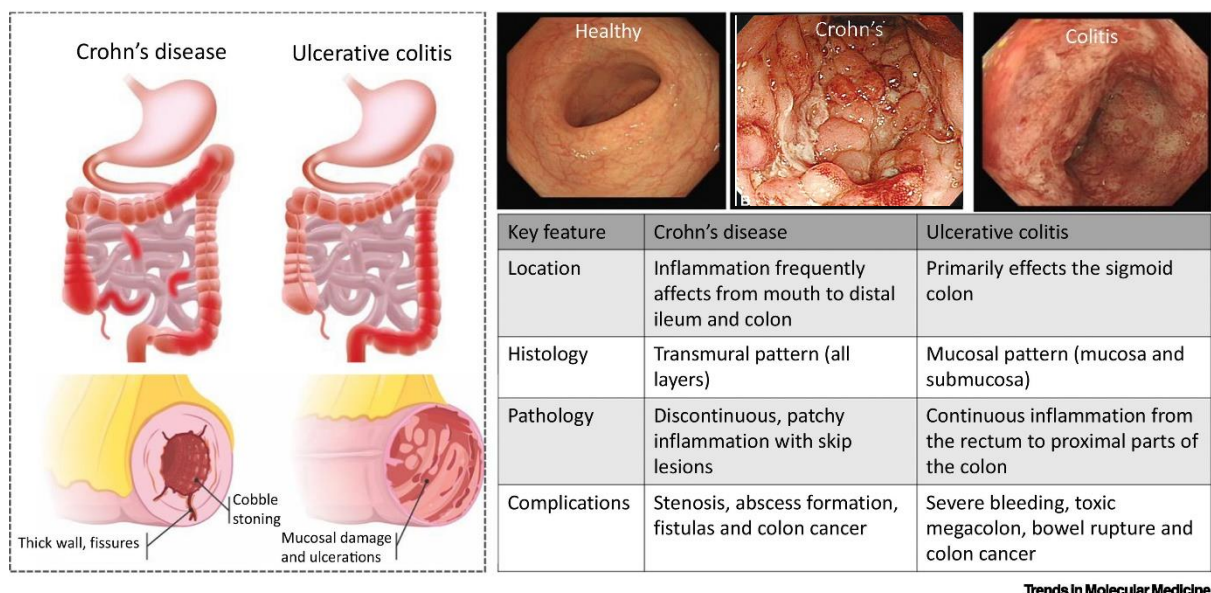


Figure 1.1: Clinical and endoscopic features of IBD.

(Source: Kotla NG and Rochev Y. *IBD disease-modifying therapies: insights from emerging therapeutics*).<sup>(14)</sup>

## 1.2 Epidemiology

A clinical syndrome of chronic bloody diarrhoea has been described since ancient times and is mentioned by Hippocrates<sup>(15)</sup> however, the report generally accepted as the first description of IBD is that of Matthew Baille in 1793.<sup>(16, 17)</sup> The term 'Ulcerative Colitis' was first used in a report by Sir Samuel Wilks in 1859.<sup>(18)</sup>

Since the latter half of the twentieth century the incidence of IBD has been increasing globally, particularly in northern industrialised nations.<sup>(19)</sup> In Western Europe, the incidence of CD ranges from 1.85–10.5/100,000 person-years and for UC from 1.9–17.2 per 100,000 person-years, with a prevalence of 28.2–322.0 per 100,000 population and 43.1–412.0 per 100,000 population, respectively.<sup>(20)</sup> In North America, the prevalence of CD lies between 44–201 cases per 100,000 population,<sup>(21, 22)</sup> while the reported prevalence of UC ranges from 37.5–238 cases per 100,000 population.<sup>(22)</sup> In a 2018 population-based study of primary care patients in the United Kingdom (UK), the prevalence of CD and UC was 279 per 100,000 population and 397 per 100,000 population respectively.<sup>(23)</sup> The incidence of IBD has also increased in newly industrialised population centres including in Africa, Asia and South America.<sup>(20)</sup> Epidemiological data from the Yunnan province,

China, showed that from 2000 to 2013 the incidence of IBD (both CD and UC) increased significantly and correlated with increasing urbanisation. (24) It is hypothesised that this change could be due to alterations in, dietary habits, obesity, and gut microbiota.

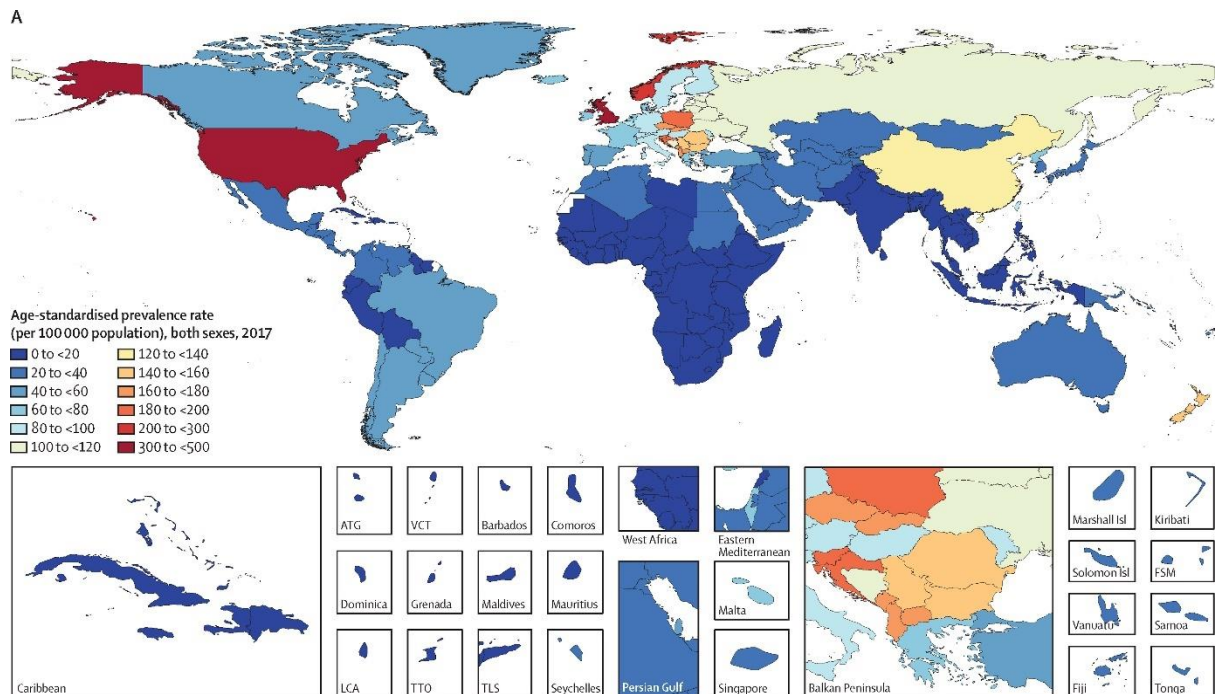


Figure 1.2: Age-standardised prevalence rate of IBD per 100,000 population. (Source: adapted from 'The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017').(25)

IBD more commonly affects females than males with a global prevalence of 93.8 [range 87.8–100.0] per 100,000 population in females and 75.0 [range 70.3–79.7] per 100,000 population in males.(25)

It is most commonly diagnosed in the second to fourth decade of life.(23, 26-28) There is evidence for a bimodal age distribution with registry studies and hospitalisation data showing a second smaller peak of diagnoses in the sixth and seventh decades of life, (29-32). Jeuring et al., in a Dutch population-based study reported that 10% of CD and 25% of UC are diagnosed in those aged 60 years and older.(33) However, 7–20% of IBD cases present in the paediatric population.(28, 34, 35)

In recent times, an increase in the incidence in children and adolescents has been noted both in Ireland(36) and globally.(37, 38) These early presentations can have significant consequences during the most productive time in a person's life leading to absences from education and work,

increased medical costs and lost earnings. One study assessing the impact of CD in the United States of America (US) calculated that the average person with CD incurs incremental costs of \$8,023 (€7,128) annually which represents an annual \$3.48 (€3.09) billion dollar loss to the economy.(39) The average lifetime cost for a US patient has been estimated at \$416,352 (€370,890) for CD and \$230,102 (€204,982) for UC.(40) The Irish Society of Crohn's and Colitis investigated the impact of IBD on an Irish population using a financial questionnaire distributed to their members in 2015. Information was sought regarding GP and specialist clinic appointments, and medication related charges. The overall annual spend per IBD patient was 1,154 euro for public patients and 3,111 euro for private patients. On average 17 sick days per patient were required annually.(41) Given the increasing prevalence of the disease and the significant economic impact, there has been a concerted effort to develop new and more effective therapies and to understand not only the pharmacokinetics but also the pharmacodynamics of novel agents and how they act within the local tissue environment. Furthering our understanding of the underlying inflammatory and immune mediated processes is key to enabling therapeutic progress and the development of a precision medicine approach for IBD.

### 1.3 Immunology of IBD

The exact cause of IBD remains elusive. It is thought to be due to dysregulation of the immune system resulting from loss of the barrier function of the gut mucosa due to a combination of environmental, genetic, and microbiotic factors. Genome wide association studies have identified a number of genetic loci variants of which are associated with a greater risk of developing UC, CD, or both. Many of these genetic loci code for proteins or receptors that have subsequently been therapeutic targets, including Janus kinase/signal transducers and activators of transcription (JAK-STAT), Interleukin (IL)-23R, and IL-12B among others.(42)

The intestinal epithelium acts as the primary barrier between the luminal contents of the gastrointestinal (GI) tract and the underlying tissue components and the inflammatory system. It is

the largest mucosal surface in the body. It is composed of different specialised subtypes of cells, all working together to absorb nutrition, water, and regulate electrolytes, while excluding potentially pathogenic or immunogenic material. The Paneth cells, goblet cells, and columnar epithelium of the mucosa are linked with tight junctions. Goblet cells and secretory cells create the mucus barrier that covers the lumen-exposed epithelial cells which form the mechanical barrier to larger particles and intact bacteria. The mucus barrier also allows for concentration and retention of antimicrobial peptides. Mice lacking a major mucin protein (MUC2) develop colitis spontaneously.(43) Paneth cells, present in the epithelial crypts of the small intestinal secrete antimicrobial peptides (AMPs), such as lysozyme C and phospholipases. In CD patients AMPs can be defective.(44)

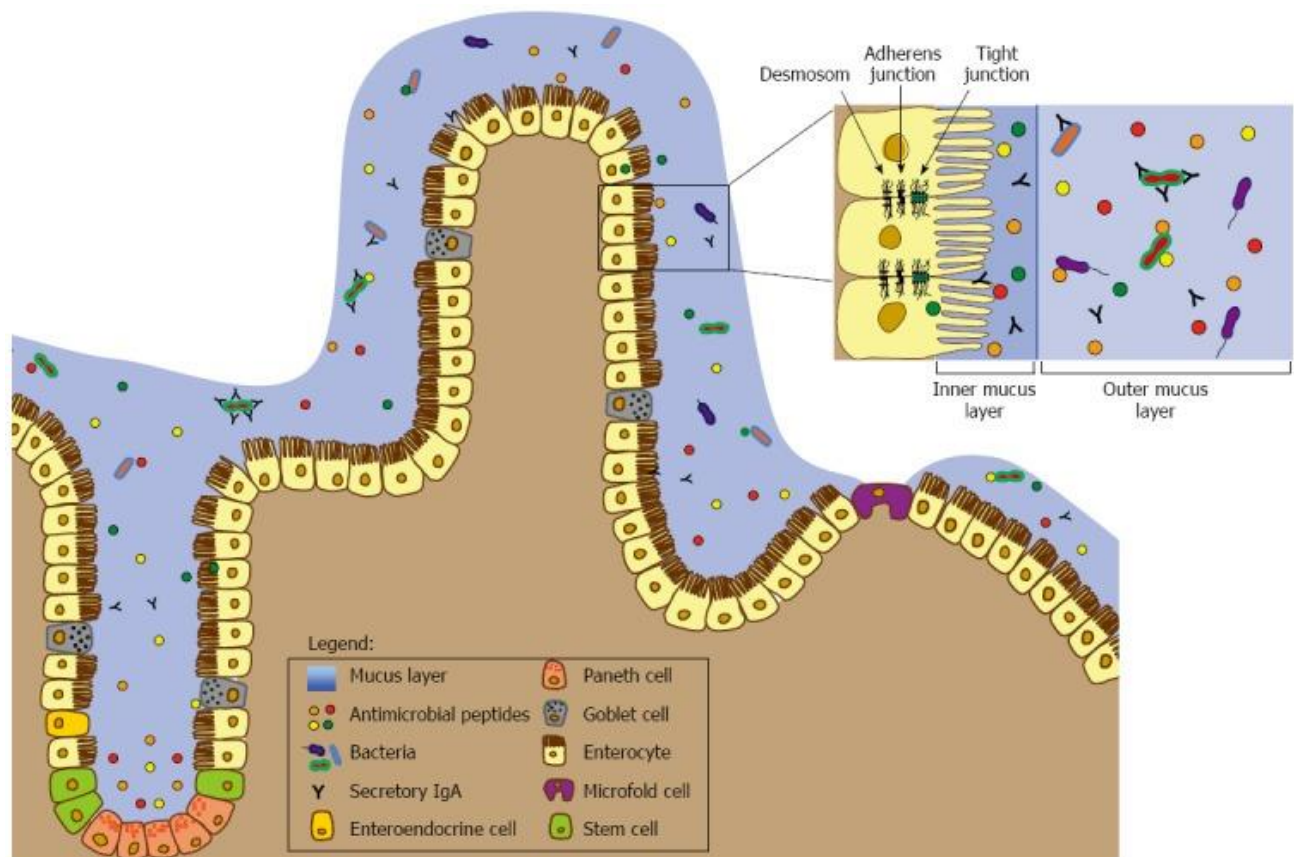


Figure 1.3: Defence mechanisms of the healthy intestinal mucosal barrier. (Source: Antoni L et al. *Intestinal barrier in inflammatory bowel disease*).<sup>(45)</sup>

Tight mucosal junctions are the complexes that seal the epithelium from the luminal contents. They act to regulate permeability of the intestinal barrier and water and electrolyte absorption. Inflammatory cytokines such as tumour necrosis factor (TNF) and interferon-gamma (IFN- $\gamma$ ) increase the permeability of these complexes leading to loss of the barrier function. Increased intestinal permeability may be caused by the significant inflammation in IBD however in quiescent IBD this impaired barrier function is still evident, and genetic loci associated with epithelial barrier function have been associated with UC. This dysregulation of the intestinal function may be a critical factor in IBD pathogenesis.

The innate immune cells of the intestine are essential in maintaining effective gut health. Macrophages, dendritic cells, natural killer cells and innate lymphoid cells act together to maintain the mucosal innate immune response. Both macrophages and dendritic cells act as antigen presenting cells (APC), binding antigens with Pattern Recognition Receptors (PRRs), such as Toll Like Receptors (TLRs) and Nucleotide-binding Oligomerisation Domain (NOD): like receptors. These cells act as the link between the innate and adaptive immune system. When certain Pathogen Associated Molecular Patterns (PAMPs) are recognised by PRRs these APCs activate the adaptive immune system through release of cytokines and binding of T Cells to APC Complex. Specialized APCs, called Microfold (M) Cells, also exist in the intestinal epithelium. These M Cells transmit antigens from the small intestinal lumen to lymphoid structures in the sub mucosa, the Peyer's patches, where they are involved in regulating immune response.(46)

As there is an element of immune tolerance in the gut, necessary because of its contact with the luminal contents, gut macrophages lack CD14, a membrane-bound protein used in the detection of the bacterial PAMP, LipoPolySaccharide (LPS), and have decreased phagocytic and chemotactic activity. Compared with the healthy gut, in CD, gut macrophages secrete more cytokines and have increased phagocytotic activity with increased production of IL-1 $\beta$ .(47) Gut dendritic cells also have higher concentration of PRR in patients with IBD that lead to increased production of the pro-inflammatory cytokines, IL-6 and IL-12.(48) In mouse models, blockage of the dendritic APC-Cell

complex ameliorates colitis.(49) Natural killer cells and natural killer T-cells contribute to the gut innate immune system primarily through activation of apoptosis in infected or cancerous cells. In IBD higher levels of inflammatory molecules are expressed by these cells.(50) The clinical severity of disease in patients with CD or UC correlates with TNF- $\alpha$  levels.

Antigen presenting cells transport antigens to Gut Associated Lymphoid Tissue (GALT) where they interact leading to maturation and activation of the adaptive immune system. GALT is comprised of a collection of lymphoid structures including Peyer's patches, lymphoid follicles, the appendix, tonsils, and smaller lymphoid tissue aggregates. Mucosal lymphocytes which originate from the bone marrow are primed in GALT and locally in the gut lamina propria. Naïve T cells undergo differentiation into Th1, Th2, Th17 and Treg Cells depending on their interaction with various cytokines. The activated lymphocytes express the  $\alpha_4\beta_7$  integrin which in turn can bind to the Mucosal Addressin Cell Adhesin Molecule 1(MAdCAM-1) present on intestinal endothelial cells, thus facilitating gut migration.

CD4+ cells, found in abundance in biopsies of inflamed CD and UC tissue, undoubtedly play a role in IBD. Anti-CD4 monoclonal antibody was one of the early monoclonal antibodies trialled in treatment of CD.(51) Higher numbers of peripheral blood CD4+ cells are found in patients with IBD compared to healthy controls, with a greater proportion being activated cells.(52). Lower CD4+ cell counts have been reported to correlate with higher remission rates in patients with HIV infection who also have IBD,(53) however a recent metanalysis was unable to confirm this finding.(54) CD4+ Cells can differentiate into TH1 cells, TH2 cells, TH17 cells, and Treg cells.

T helper 1 (Th1) cells are CD4+ lymphocytes produced in response to maturation in presence of IL-12 acting through the JAK2–STAT4 cell signalling pathway. Th-1 cells tend to be pro-inflammatory. They are activated in response to intracellular pathogens in the healthy immune system producing IFN- $\gamma$ , TNF- $\alpha$ , IL-2 and IL-12. This in turn leads to activation of the NK Cells, macrophages and CD8 positive cytotoxic (CD8+) T Cells that are necessary to combat infection. However, an excessive response with uncontrolled inflammation is detrimental.

Many IBD therapeutic agents work either through inhibition of cytokines leading to Th1 maturation, e.g., ustekinumab targeting IL-12 and IL-23, or target the inflammatory cytokines produced by Th1 cells e.g., the anti-TNF agents.(55-64)

In the healthy immune system T helper 2 (Th2) cells and their effector cytokines are involved in host protection against extracellular parasites, bacteria, allergens and toxins. Maturation of naïve T cells in the presence of IL-4 is associated with Th2 differentiation. IL-25, IL-33 and Thymic Stromal Lymphopoietin (TSLP), all of which are produced following epithelial injury, are also linked with Th2 cell differentiation.(65)

Following activation of the STAT6 signalling molecule by IL-4 signalling, the GATA3 transcription factor is activated which leads to further Th2 differentiation and inhibition of Th1 cells. The GATA3 transcription factor is considered a lineage defining factor in Th2 cells.(66) Th2 cells produce IL-5, IL-13 and IL-4. IL-4 acts to inhibit the differentiation into Th1 cells. For health, a well-balanced Th1/Th2 response is optimum. Excessive responses in either direction can be associated with a variety of different disorders.

Ulcerative colitis is considered a disease which exhibits a Type 2 inflammatory response characterised by IL-5 and IL-13 production.(67-69) Biopsies of inflamed UC mucosa had higher concentrations of IL-5 and IL-13 mRNA than that of either healthy control mucosa or in inflamed CD mucosa.(69)

T helper 17 (Th17) cells are also part of the host defence system. While they are important in protection against extracellular infections, they are involved in the pathogenesis of some autoimmune diseases. Differentiation of naïve cells into Th17 cell is primarily controlled by transforming growth factor- $\beta$  (TGF- $\beta$ ), IL-6, IL-21 and IL-23.(70-72) The cells were so named because of their production of IL-17 cytokines including IL-17A and IL-17F that induce expression of GCSF, chemokines and antimicrobial peptides.(73) Th17 cells also express other cytokines including IL-21, IL-22, and IL-23 and are subject to autocrine differentiation. Increased circulating Th17 cells and

their effector cytokines have been found in multiple conditions including RA, SLE, Sjogren's syndrome, psoriasis, and asthma.(74) The effector cytokines are significantly associated with IBD and are present in large quantities in the inflamed gut mucosa.(75) Th17 produced cytokines, IL-17A and IL-17F, are upregulated in both the serum and in the mucosa of patients with IBD compared to controls.(76) Biopsies from inflamed mucosa of patients with CD have higher concentrations of IL-17, IL-23 and IL-32 mRNA indicative of a Th17 cell inflammatory response. This is not found in UC.(69)

Regulatory T (Treg) cells are so named because of their effect on immune tolerance. First described in 2000, they are characterised as CD4+CD25+ positive T cells that produce the transcription factor Forkhead box P3 (FoxP3). They consist of about 5-10% of CD4+ T Cells that undergo differentiation from naïve T cells in the presence of IL-2.(46) Reduced levels of Treg cells are present in the peripheral blood of patients with IBD compared to controls.(77) In IBD, increased numbers of circulating Treg cells have been reported within 14 days of anti-TNF IBD therapy initiation.(78) Tregs cells can be broadly categorised in two groups. Natural Treg (nTreg) cells differentiate in the thymus and are mainly involved in regulating immune tolerance to endogenous antigens. Induced T regulatory (iTreg) cells arise from naïve T cells following their interaction with APCs at sites of mucosal interfaces in the presence of IL-2 and TGF- $\beta$ . In the gut mucosa this can happen in GALT. T reg cells inhibit inflammation through inhibition of APCs or direct effector cells. They produce high levels of IL-10, which acts as an autocrine proliferator of Treg cells and can inhibit TNF- $\alpha$  and IL-1 $\beta$  production from myeloid cells.(79) Genome wide association studies have linked several genetic loci associated with IL-10 to CD.(42) Treg cells also inhibit inflammation through direct interaction with target inflammatory cells; they can down regulate APC activation through expression of cell surface receptors (CTLA4 and LAG-3).(46, 80)

Activation of naïve T cells in GALT also results in up-regulation of the integrin receptors that allows for cellular migration to the required sites of inflammation. These integrins consist of heterodimers with an  $\alpha$ -subunit of which there are 18 variations and a  $\beta$ -subunit of which there are eight different

variants. Varying subunit combinations permits the differential cell binding that allows for migration to specific tissues. Expression of the  $\alpha_4\beta_7$  integrin facilitates colonic migration whereas for the small bowel migration  $\alpha_4\beta_7$ ,  $\alpha_4\beta_1$ , and  $\beta_2$  integrins are expressed. In gut lymphocyte migration these integrins bind to Cell Adhesion Molecules (CAM) which are expressed in High Endothelial Venules (HEV) of the gut. Intracellular CAM (ICAM)-1, Vascular CAM (VCAM)-1, and MAdCAM-1, are all involved in integrin-dependent lymphocyte migration to the gut. However, only MAdCAM-1 is considered to be largely gut-specific.(81)

High endothelial venules associated with GALT expresses MAdCAM-1 and other CAMs.  $\alpha_4\beta_7$  binds to MAdCAM-1,  $\alpha_4\beta_1$  binds to VCAM-1, and  $\beta_2$  heteromers bind to ICAM. The low affinity binding of L-selectin and integrins to CAM expressed on the endothelium of blood vessels permits circulating lymphocytes to tether and roll along the blood vessel endothelium. This slowing down of the T cells along endothelium expressing these ligands gives time for chemokines to exert their effects.. Chemokines, such as IP-10 and MIP- $\alpha$ , activate the cell and the integrin facilitating more adherent binding with their ligand and enabling lymphocyte migration across the endothelium. The binding of activated integrins to their ligands allows for cytoskeletal rearrangement and extravasation across the endothelial barrier.

## 1.4 Treatment of IBD

### 1.4.1 5-amino salicylic acid preparations (5-ASA)

Early treatment of IBD was not standardised and ranged from that which was likely mildly beneficial; dietary interventions such as low residue high protein diet and vitamin supplementation, to the frankly harmful e.g., rectal instillations of hydrogen peroxide, silver nitrate, or tannic acid.(82) However in the 1930s, the hypothesis that streptococcal infection of connective tissue was responsible for both rheumatoid arthritis (RA) and UC found favour and, as a result, an antibacterial drug that was capable concentrating in connective tissue was developed as a treatment for RA. That drug was sulphasalazine. Sulphasalazine consists of two components, sulphapyridine and 5-aminosalicylic acid (5-ASA), was first manufactured in 1938.(83) It was initially used in the treatment of RA. Given the limited treatment options available at that time, following

a nine patient observational UC study suggesting that it would also be effective,(83) it became the agent of choice for treatment of UC. (84)

It was not until 1962 that the first placebo-controlled trial of sulphasalazine was conducted. That study showed benefit of a high dose (4g/day) sulphasalazine in inducing disease remission, although not without a cost in terms of side effects.(85) In 1965, a maintenance dose of 2g/day for up to a year was shown to be effective and was better tolerated than the higher 4g dose.(86) The value of long-term maintenance therapy for UC was generally accepted following a publication by Dissanayake and Truelove in 1973. In a placebo-controlled trial they showed significant benefit for patients who continued therapy; 55% of placebo recipients relapsed compared with 12% of sulphasalazine recipients over the five-year period.(87)

The active ingredient of sulphasalazine was subsequently discovered by comparing the effect of each of the two drug components, given by enema, on inflamed colon. It was not the sulphapyridine but rather the 5-ASA component that was found to be effective.(88) This led to the development of other ASA drugs, mesalazine and olsalazine, as UC treatment.

Prior to 1960 there was no adequate distinction made between colonic CD and UC.(10) Thus , many of the early 5-ASA UC trials likely included some patients with CD. From 1990-2010, over half of all UK patients with CD were prescribed maintenance 5-ASA therapy.(89) Now, there is only a limited role for 5-ASA in CD. Based on a 2016 Cochrane systematic review that showed no benefit of 5-ASA agents in CD,(90) most current guidelines recommend against their use for induction or maintenance therapy.(12, 91) Based on two early randomised controlled trials (RCT) that in subgroup analysis showed benefit of sulphasalazine limited to colonic CD,(92, 93) the American College of Gastroenterology (ACG) included a recommendation that sulphasalazine, but not melasalazine or olsalazine, could be used for treatment of patients with mild to moderately active colonic CD but not in those with isolated small bowel disease. ACG considered it to be of limited benefit in preventing postoperative Crohn's disease however based on moderate evidence cite it as an option for patients with isolated ileal resection and no risk factors for recurrence.(94) The

European Crohn's and Colitis Organisation however recommend against using it in either induction or maintenance of Crohn's Disease.(91)

#### 1.4.2 Glucocorticoids

The first clinical trial to assess glucocorticoids as treatment for acute UC was carried out by Truelove and Witts in 1955.(95, 96) They assessed the response to cortisone, given daily for six weeks at an initial dose of 100mg/day for induction of remission, in patients having a UC flare. They concluded that patients receiving cortisone *"enjoyed a clear advantage"*. Subsequent clinical trials discovered that corticosteroids were more effective than sulphasalazine in induction of remission in UC.(97, 98) In the 1970s, the combination of intravenous (IV) steroids given together with steroids delivered by enema proved effective in acute severe UC.(99) A systematic review of patients receiving IV glucocorticoids for acute severe UC that required hospitalisation showed a short term colectomy (defined as colectomy during that hospital admission) rate of 27%.(100)

The first RCT to give evidence of corticosteroid efficacy in CD treatment was published in 1979. A US multi-centre study examined the response of active and quiescent CD to prednisolone, sulphasalazine or azathioprine (AZA). Both prednisolone and sulphasalazine were more effective than placebo in induction of remission however the benefit of sulphasalazine was mainly seen in those with colonic disease.(92) In 1984, Malchow et al. reported the results of the European Cooperative CD study and found that overall steroids were the most effective agents for CD. The combination of methylprednisolone and sulfasalazine was the most effective in untreated patients and when disease was confined to the colon.(93)

Short-term treatment with systemic corticosteroids for acute flares in moderate to severe colitis has become the standard of care for IBD.(12, 91, 94, 101) However, the long-term side effects of steroid therapy can be problematic and include Cushing's syndrome, acne, infection (increased risk of abdominal and pelvic abscesses in patients with CD), ecchymoses, hypertension, diabetes mellitus, osteoporosis, cataracts, glaucoma, and growth failure in children.(102)

It was not until 1994 that a non-systemic based corticosteroid therapy for IBD was released. Budesonide was originally developed as an inhaled steroid for treatment of inflammatory airway disease.(103) Of absorbed budesonide, 90% is metabolised by first pass metabolism in the liver.(104, 105). Consequently, it is associated with significantly less side effects compared to a similar dose of a systemic corticosteroid (33% vs 55%).(106) However due to instability in gastric acid, often it does not survive transit through the stomach. To counteract this and ensure delivery to the ileum, sustained enteric coated pellets, containing a layer of budesonide in ethylcellulose with a sugar core, were developed.(107) This ensures adequate delivery to the ileum and caecum, making it an effective treatment for ileocaecal CD.(108)

Although it is effective in inducing remission in ileocaecal CD and has less side effects, a Cochrane review reported that it is not as effective as systemic corticosteroids for treatment of CD.(109) Thus, it is not recommended for treatment of severe flares of ileocaecal CD.(8, 12, 91, 94)

Ileal preparations of budesonide have been shown to be ineffective in UC as the compound does not reach the area of most disease activity; the rectum.(110) Rectal budesonide preparations were developed for UC and are effective for proctosigmoiditis and proctitis,(111, 112) however, not more so than a 5-ASA enema.(113)

In 2012 a colonic release oral preparation of budesonide (Multi-matrix Budesonide (Budesonide-MMX) entered clinical trials.(114, 115) Subsequent sub-group analysis showed that while budesonide-MMX is most effective in left sided colitis it is not effective in pancolitis.(116) Budesonide-MMX is recommended for use in UC however there is little data for its use in colonic CD.(12)

#### 1.4.3 Thiopurines

Following the success of sulphasalazine that was initially developed as a treatment for RA, other immunosuppressive therapies effective in RA were trialled. Thiopurines, that include 6-mercaptopurine (6MP) and azathioprine (AZA), are antimetabolites used in the treatment of other inflammatory diseases, including systemic lupus and RA.(117)

Thiopurines are metabolized by the thiopurine methyltransferase (TMPT) pathway to produce a purine antagonist thereby inhibiting DNA and RNA synthesis. This inhibits cell proliferation, most effectively in rapidly dividing cells such as inflammatory cells.

Thiopurines have been in use in IBD since 1962,(118) however the early experience was not promising. Azathioprine was found to be inferior to sulphasalazine.(119) The first RCTs of thiopurines in CD were in the 1970s.(120-122) Most of the studies had small sample sizes and heterogeneous endpoints. Summer et al. in the largest CD study of the time with a well-defined endpoint included 136 patients and used a CD Activity Index (CAI) score of less than 150 to indicate remission.(92) There was a slightly higher rate of remission in AZA recipients compared with placebo (36% vs 26%), however, the difference was not statistically significant.(92)

During the same time period, small RCTs compared the induction of remission in patients with UC proctocolitis by AZA compared with either sulphasalazine(123) or corticosteroids(124) and found no benefit. This early lack of effectiveness was confirmed in a subsequent meta-analysis that did not find any significant benefit for AZA as induction therapy of UC(125) or CD.(126) Thus, thiopurines are not recommended as induction therapy of either CD or UC.(12, 91, 94, 101)

Thiopurines have proven much more effective as maintenance therapy for CD. The first RCT trial of AZA for CD maintenance was reported in 1971. This study, with very low numbers (five in each arm), looked at patients following induction of remission by prednisolone. At week 24, four of five AZA recipients remained in remission compared with two of five placebo recipients.(122) These findings were replicated many larger studies.(127) There is less evidence to support the use of thiopurines in the treatment of fistulising CD. However, in one meta-analysis, healing of fistulising CD was observed in 54% of AZA or 6MP recipients compared with 21% of those who received a placebo.(128)

Early trials of thiopurines for maintenance therapy of UC were not promising. In 1974, of 80 hospitalised patients with UC, no statistical difference in outcome between 40 patients receiving

2.5mg/kg AZA daily compared to 40 placebo recipients was found although a trend towards improvement with AZA was reported.(124) In 1992, Hawthorne et al. reported that AZA was significantly better than placebo in maintenance of remission in corticosteroid dependant UC, with lower relapse rates in AZA recipients compared with placebo (12/33 vs 20/34 respectively,  $p=0.01$ , HR 0.5, 95% CI 0.25-1.0).(129) A 2016 Cochrane review that included trials of at least 12 months duration confirmed the benefit of AZA compared with placebo for the maintenance of remission in UC and that it may be an effective option for those intolerant of mesalazine or sulphasalazine.(130)

Thiopurines have long been used in the treatment of UC, however, about 20% of patients on AZA experienced an adverse event.(131) This could be due to gastrointestinal upset although other side effects including pancreatitis, hepatotoxicity, and bone marrow suppression can result in treatment cessation. Thiopurines are also associated with serious but rare adverse events including an increased risk of skin cancer(132) and T-Cell lymphoma(133) with a 4-5 times increased risk of lymphoma reported.(134)

The safe metabolism of thiopurines relies on presence of the enzyme ThioPurine MethylTransferase (TPMT), the activity level of which varies in the population. Although 90% of the population has normal activity, complete TPMT deficiency occurs in 0.3% of the population. Without adequate levels of TPMT, thiopurines are metabolized into 6-methylmercaptopurine (6MMP) which is hepatotoxic(135) and bone marrow toxic.(136) It is now a standard of care to assay levels of TPMT in all patients with IBD before starting therapy with a thiopurine.

#### 1.4.4 Methotrexate

Like thiopurines, methotrexate (MTX) was developed as an anti-cancer drug, first targeting leukaemia, then later established as a treatment for solid organ cancers. It works as an antimetabolite, competitively inhibiting folate metabolism. This is essential for mitosis and purine synthesis. Like thiopurines, methotrexate targets rapidly dividing cells including neoplastic and inflammatory cells. MTX was shown to be effective in rheumatological conditions in the 1950s and 1960s. However, it was not until 1989 that the first report of MTX's use in IBD treatment was

published. Kozarex et al., included 14 patients with CD and seven with UC with treatment refractory IBD.(137) An induction course of 25mg MTX given intramuscularly (IM) once weekly for 12 weeks and followed by weaning to a minimum dose of 7.5mg weekly. Eleven of 14 patients with CD and five of seven with UC patients showed clinical response,  $p \leq 0.007$ . Mucosal healing occurring in five of 11 patients with CD but in none of the patients with UC. Larger placebo- controlled trials of CD followed in the 1990s and early 2000s. In corticosteroid dependant CD, 39% patients achieved remission following a 12 week induction with 25mg MTX IM per week compared with only 19% of placebo recipients,  $p=0.025$ .(138) A 15mg weekly oral dose was shown to maintain remission in 65% of MTX recipients compared with 39% of placebo recipients,  $p=0.04$ .(139) A large multicentre RCT of lower dose maintenance therapy (oral MTX 12.5mg, once weekly) showed no significant benefit compared with placebo over a nine month period for treatment of CD.(140)

Two relatively recent clinical trials, 2015(141) and 2018(142), showed that in corticosteroid dependant UC, MTX is neither effective in inducing remission nor useful in maintaining corticosteroid-free remission.(142) Like thiopurines, MTX is not without the potential for significant adverse reactions. Approximately 20–33% of patients discontinue therapy due to adverse events.(143) Nausea, reported in up to 25% of patients is the most common side effect. Rarer but more serious side effects include hepatotoxicity, bone marrow suppression, hypersensitivity pneumonitis, and infectious complications of immunosuppression.(143)

#### 1.4.5 Ciclosporin

Although MTX is effective in inducing remission in steroid dependant CD, similar success was not found for UC. Intravenous steroids were the only available agents to treat an acute UC flare, failing which the only option was surgery. There had been very little progress in management of acute severe UC flares during the preceding 40 years. Due to the success of MTX in CD, attention turned to other immunosuppressives as potential therapies. Ciclosporin, is an immunosuppressive agent used in solid organ transplantation to prevent rejection. It works by binding to a cytoplasmic protein, cyclophilin, and leads to selective inhibition of calcineurin. Calcineurin is a regulatory factor

involved in control of many inflammatory genes including IL-2, IL-4, TNF- $\alpha$ , GCSF, and IFN- $\gamma$ . Ciclosporin treatment leads to marked reduction in T lymphocyte production. While in 1989 a small RCT of ciclosporin in CD in reported some benefit, (144) this was not replicated in larger trials(145-147) and a trend towards harm in two studies was noted.(146, 147) There was some observational evidence to support its use in the treatment of fistulising CD, however relapse rates were high following drug discontinuation.(148, 149) As a result ciclosporin was generally abandoned as a treatment for CD. However a lack of any good alternative coupled with the demand for rescue treatment for steroid refractory severe UC led to further clinical trials of ciclosporin in the 1990s.

In the first RCT of 20 patients with acute severe UC, Lichtiger et al. reported that 4mg/kg/day of ciclosporin was effective for patients with refractory UC and achieved a short-term improvement in 76–85% of patients.(150) Although it was initially planned to enrol 40 patients because of the reported benefit, the trial was halted prematurely by the by the drug safety and monitoring committee after the first 20 patients completed treatment.(151) Subsequent studies have shown a 2mg/kg/day dose is equally effective as the higher dose.(152, 153) For those with acute severe corticosteroid refractory UC, short term colectomy can be avoided in more than two thirds of ciclosporin recipients (91, 150, 154) with a median time to clinical response of four days.(155) Ciclosporin has a narrow therapeutic index and therapy must be carefully monitored. Significant side effects include renal insufficiency, hypertension, hypomagnesemia, seizures, and increased risk of opportunistic infection.(156, 157). An increase in long term mortality has also been reported.(157). Over the seven years following ciclosporin induction, 58%-88% of UC patients required colectomy.(156, 158) Current ciclosporin based induction regimens recommend a gradual weaning of ciclosporin and continued maintenance therapy with a substitute agent.(101) Successful transition to an oral thiopurine can significantly reduce the requirement for colectomy. Of 71 patients who received ciclosporin as induction therapy and with a mean follow up of 1.5 years, 60 responded of whom 26 were transitioned to 6-MP. Only one patient who was successfully

transitioned to 6-MP required colectomy whereas 26/34 who were not transitioned required colectomy  $p < 0.001$ .(159)

#### 1.4.6 Biologic Therapy for IBD

Biologic medications contain an active substance; hormones, proteins, or immunoglobulins, that are derived from a biological source such as a cell culture or living organism. Biologics are well established in clinical practice for treating conditions such as diabetes (e.g., insulin) and other hormonal conditions (e.g., PTH, L-Thyroxine).(160) With the development of the next generation of biologic agents consisting of monoclonal antibodies designed to neutralise specific pro-inflammatory proteins, the goal of more targeted therapy for a wide range of conditions could be realised.

##### 1.4.6.1 Anti-Tumour Necrosis Factor Therapies

In the late 1990s, further insight into the pathways of inflammation in IBD led to development of targeted therapies designed to selectively inhibit specific key inflammatory proteins. Tumour Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), a cytokine initially identified as a protein triggering apoptosis in cancer cells(161) was subsequently found to be elevated in a variety of inflammatory states including bacteraemia, septic shock, graft versus host disease, and inflammatory rheumatological conditions.(162) In 1992, following discovery of elevated TNF- $\alpha$  levels in the synovial fluid and tissues of patients with RA, TNF- $\alpha$  was identified as an attractive therapeutic target for RA.(163, 164)

Mouse derived monoclonal antibodies directed against TNF- $\alpha$  had previously been trialled in humans for treatment of shock,(165) however their continued development was limited due to an associated allergic inflammatory/hypersensitivity reaction that developed when re-administered. New chimeric antibodies consisting of a mouse antigen binding region and a human Fc region were developed as a means to reduce potential immunogenicity and overcome this limitation.(166)

##### 1.4.6.1.1 Infliximab

The first chimeric anti-TNF monoclonal antibody developed in 1993 by Knight et al. consisted of a humanised mouse IgG1 antibody wherein the murine antigen binding regions were retained while

the fixed regions were replaced by their human counterparts.(162) It was called infliximab (IFX) with the suffix “-liximab” denoting an immunomodulating (li) chimeric (xi) monoclonal antibody (mab). It was first reported as a very successful treatment for RA in the 1990s.(167) The first case reports of its use in CD appeared in 1993.(168)

The pivotal large scale RCT of IFX for CD, ACCENT I, was not published for another nine years. ACCENT I evaluated 5mg/kg and 10mg/kg of IFX given at weeks 0, 2, and 6, in induction followed by an infusion every eight weeks as maintenance and compared the regimen with placebo. Although there was no difference between the doses, a significantly higher percentage of IFX recipients were in remission at weeks 30 and 54.(57) The ACCENT II trial focused on patients with fistulising disease. This large multicentred trial included 306 patients with perianal fistulae of at least three months duration. All patients received standard induction of 5mg/kg IFX, as described in the ACCENT I trial, after which they were randomised to receive 5mg/kg IFX every eight weeks or placebo with the option of increasing the dose to 10mg/kg for any patient failing therapy. Overall, 64% of patients responded defined as a reduction of open draining fistulae. Those receiving IFX maintenance therapy had a significant reduction in hospital admissions and in requirement for surgery.(169) This was the first prospective trial designed to specifically look at fistulising CD.(91)

Infliximab was found to be particularly useful in treatment of acute severe UC(55) and provided an alternative to ciclosporin.(155) The first pilot study of 11 patients with UC published in 2001 reported efficacy in treatment of acute severe steroid refractory UC.(55) This was subsequently replicated in several other open label and blinded studies.(56, 170-177) The key papers showing efficacy in both induction and maintenance were the Active Ulcerative Colitis Trials (ACT1 and ACT2).(178) IFX was found to be non-inferior to ciclosporin and provided the option of continuing IFX for maintenance therapy.(155) One of the main drawbacks of IFX is the requirement for IV administration. The requirement for infusion suite beds places significant stress on limited hospital resources, and the requirement for patients to attend hospital for infusions takes time,

commitment, and can unnecessarily expose immunosuppressed patients to a hospital environment.

In 2019, a subcutaneous IFX formulation was developed and approved for IBD, by which time many other subcutaneous biologic therapeutic options had also become available.(179, 180) Using an IFX biosimilar, Schreiber et al. showed that patients receiving subcutaneous IFX had higher trough IFX concentrations than those receiving IV therapy. Both methods of administration had similar remission rates at week 30.(125) Although IFX is effective for both CD and UC there remains a high rate of disease relapse over time with only 40% of patient achieving sustained remission at one year.

#### 1.4.6.1.2 Adalimumab

Following the success of IFX, the search began for other anti-TNF biologic agents that might be even more effective for IBD. Adalimumab (ADA) was first tested and approved for treatment of RA.(181) It is a fully humanised monoclonal antibody, suitable for subcutaneous infusion, that was thought to be less immunogenic than the chimeric IFX.

The first dose finding study of ADL in IBD, the Clinical assessment of Adalimumab Safety and efficacy Studied as Induction therapy in CD (CLASSIC–I) trial, included 299 patients with moderate to severe CD. Based on the CLASSIC-I trial data, the investigators considered that a first dose of 160 mg ADA SC followed by 80mg SC at week 2 was optimum for induction. The week 4 remission rate in ADL recipients was 36%, compared with 12% in those receiving placebo.(182) Patients in remission at week 4 were again randomised, this time to continue to either 40mg ADA weekly, 40mg ADA every other week, or placebo as part of the CLASSIC–II trial and clinical status was reassessed at week 52.(4) Remission rates of 83%, 79%, compared with 44%,  $p<0.05$ , were reported for those receiving ADA weekly, every other week, or placebo, respectively. This benefit of ADA was also shown in the phase III Crohn's disease trial of the fully Human antibody Adalimumab for Remission Maintenance' (CHARM). Using an induction regimen of 80mg at week 0 and 40mg at week 2, the results of ADA 40mg given weekly or given biweekly were again compared. No

significant difference between the regimens was found with remission rates of 40% and 36%, and 47% and 41% for weeks 26 and week 52 respectively. Both treatment regimens fared better than placebo with remission rates of 17% and 12% for weeks 26 and 52 respectively.(183) ADA was also shown to be effective in treatment in patient who have failed IFX.(184)

The earliest open label study assessing efficacy of ADA for UC was in 2007.(185) This non blinded study showed ADA was capable of inducing remission in patients who had failed IFX. The first major trial in UC was not available until 2011.(186) The 'Ulcerative colitis Remission and Maintenance with Adalimumab (ULTRA-1)' showed that induction of remission in patients with corticosteroid refractory moderate to severe colitis was possible using an induction regimen of 160mg ADA at week 0, 80mg two weeks later, followed by 40mg every other week, was more effective than placebo.(173) Nineteen percent of patients receiving this regimen attained remission at week 8, compared with 9% receiving placebo. A third arm in this study showed that an induction regimen of 80mg at week 0 followed by 40mg every two weeks resulted in a remission rate of 10% at week 8 and was not more effective than placebo. ULTRA-2 focused on ADA as maintenance therapy with week 52 corticosteroid free remission as its endpoint.(187) This study stratified patients according to previous anti-TNF therapy. Among TNF naïve patients week 52 remission rates were 22% and 12.4% for the treatment and placebo arms respectively. In TNF exposed patients, week 52 remission rates were lower at 10% and 3% for treatment and placebo arms respectively.

Following these trials, ADA was approved for induction and maintenance therapy of CD and UC. The currently recommended regimen for induction is 160mg at week 0 followed by 80mg week 2 and 40 mg every three weeks thereafter as maintenance therapy.

#### 1.4.6.1.3 Golimumab

The most recent anti-TNF therapy approved for IBD is golimumab (GLB). This is a fully humanised monoclonal antibody that had been approved for treatment of RA,(188) ankylosing spondylitis,(189) and psoriatic arthritis.(190) It is administered every four weeks by subcutaneous injection using a pre-filled pen. The Program of Ulcerative Colitis Research Studies Utilizing an

Investigational Treatment (PURSUIT)(58) was the first Phase III RCT to evaluate the efficacy of GLB in UC; 51% of recipients achieved an induction response. The maintenance study (PURSUIT 2) reported that 42% of induction responders maintained clinical remission at week 52.(59) While there have been a number of case series and retrospective studies reported, there has been no prospective RCT of GLB in CD, either for induction or maintenance. It is not yet authorised for use in CD.(191)

#### 1.4.6.1.4 Etanercept

One of the first anti-TNF molecules assessed for efficacy in CD was etanercept. Unlike IFX this is a not a monoclonal antibody; instead it consists of a soluble human TNF receptor that is fused with the Fc portion of a human IgG protein. It only binds soluble TNF. Etanercept is available for SC administration. It is also effective for RA(192). Early case studies of its use in CD had been promising.(193) However, when formally evaluated for the treatment of active CD it failed to perform significantly better than placebo.(194) Etanercept is neither approved nor used in the treatment of IBD.

#### 1.4.6.1.5 Certolizumab Pegol

Certolizumab, a TNF antagonist, is also available as a SC preparation. It is a humanised monoclonal TNF binding antibody that is pegylated to increase its half-life. The PRECISE study included 662 patients with CD treated with an induction dose of 400mg Certolizumab Pegol given every two weeks followed by the same 400mg dose every four weeks as maintenance. This regimen was associated with a clinical response at week 6 and 26, however the week 26 remission rates were not significantly different from placebo.(63) The PRECISE 2 trial of certolizumab as maintenance therapy for those with moderate-to-severe CD enrolled the patients who had a clinical response at week 6. With continued therapy 48% achieved clinical remission at week 26 compared with 29% in the placebo arm ( $p < 0.001$ ). (64) Certolizumab received FDA approval for treatment of CD however approval was denied by the European Medicines Agency (EMA) *“as in the study of induction treatment CIMZIA (certolizumab pegol) showed only marginal effectiveness, which was too low to*

*be relevant for patients*” and there were concerns that the duration of the maintenance therapy was too short to give information on long term side effects.

#### 1.4.6.1.6 Comparisons of Anti-TNF Therapies

A meta-analysis of RCTs examined anti-TNF treatment for induction and maintenance of CD.(195)

Ten studies; two IFX, four ADA, four certolizumab; six with induction treatment outcomes, and five with maintenance outcomes, were included with 1771 subjects undergoing induction and 1690 maintenance. The authors concluded that ADA was superior to certolizumab for induction and there was a trend towards, but not reaching, significance for IFX as superior to both ADA and certolizumab. There was no significant difference between maintenance therapies.(195)

For induction of remission in UC, a network meta-analysis that included 15 RCT in biologic naïve and seven RCT in biologic exposed patients showed that IFX was superior to both ADA and golimumab in biologic naïve patients.(196)

#### 1.4.6.1.7 Biosimilars

Unlike traditional small molecule drugs, monoclonal antibodies are large structurally complex arrangements of proteins typically derived from either cultured cells or other living organisms that are genetically altered to produce large volumes of the specific monoclonal antibodies. By comparison an aspirin molecule that contains 21 atoms, whereas IFX is a much more complex molecule containing 10–100 times as many atoms. Biologic medicines require more complex manufacturing processes to ensure a homogenous product. As a result, development of a generic compound that is an exact copy of the reference molecule is not possible. Instead of an exact generic formulation, biosimilar products are created. These are products that are highly similar and have the same mechanism of action as the reference product without any difference in potency, purity, safety, or clinical outcomes. They are developed by competing drug companies when the original product patent is expired and are generally marketed at a lesser cost.(197) Following development, biosimilar medications are required to prove equivalence with the reference medication in non-clinical and clinical studies. When this is satisfactorily established, extrapolation

to all conditions for which the reference medication is indicated is allowed thus avoiding unnecessary clinical trial repetition.(160) Currently, there are biosimilars of IFX and ADA. There has been no significant increase in treatment failure in patients who have switched from the reference medication to the biosimilar.(198) Biosimilar development has resulted in greater market competition leading to lower drug cost and innovation in treatment delivery.(199) The development of IFX available for SC use is the result of innovative biosimilar development. While the term biosimilar is generally taken to refer to the newer biologic formulations that are bioequivalent to the originator product e.g., infliximab, it should be recognised that the originator products are also technically biosimilars. As biologics are produced in living organ systems it is impossible for even the originator biologic to be identical from batch to batch. For example, the infliximab used in North America while bioequivalent is not identical to that used in Europe.(200)

#### 1.4.6.1.8 Anti-TNF therapy Summary

In summary, TNF- $\alpha$  is an important pro-inflammatory cytokine in IBD. Randomised controlled trials have demonstrated the efficacy of anti-TNF- $\alpha$  monoclonal antibodies as therapies for UC and CD. Anti-TNF therapy has significantly reduced hospital complication rate by 50% and the need for surgeries in IBD by 33–77%.(201-203) However, a significant portion of patients either do not respond to these agents or following the initial response effectiveness is lost.(58, 59, 178, 204, 205) There remains a considerable unmet need for new, alternative and more effective IBD therapies.

#### 1.4.6.2 Ustekinumab

Both IL-12 and IL-23 have been implicated in pathogenesis of inflammation in CD and UC. IL-12 has been implicated as the dominant pathway in certain mouse models of colitis. Both cytokines drive production of TNF- $\alpha$ . IL-12 drives differentiation along the Th1 pathway leading to production of IFN- $\gamma$  Gamma whereas IL-23 leads to differentiation down the Th17 pathway. Ustekinumab is the newest biologic agent to gain approval for treatment of IBD by the FDA and the EMA. It inhibits each of these pathways through binding the p40 subunit that is present in both IL-12 and IL-23.

Inhibition of IL-12/23 with ustekinumab is effective in other inflammatory diseases e.g., psoriasis and psoriatic arthritis.(206) It can be given IV or SC. An initial phase II trial of ustekinumab assigned patients to a range of IV induction regimens; a single dose of either 1, 3, 6mg/kg ustekinumab or placebo. Initial trials showed a clinical response however remission was not achieved. An induction dose of 6mg/kg had the greatest effect on disease response.(60) a further trials comparing the 6mg/kg dose to placebo in 494 anti-TNF naïve patients and 418 anti-TNF exposed patients showed that significantly more patients in the treatment arm achieved corticosteroid free remission at week 8.(61). In the anti-TNF naïve group, 40% of treated patients were in remission at week 8 compared with 20% of placebo recipients. In the anti-TNF exposed group, 21% of treated patients were in remission at week 8 and 7% of patients in the placebo arm.(61) The patients who responded to the induction therapy were then randomised to either ustekinumab 90mg SC every 8 weeks or placebo as maintenance. Fifty-three percent of anti-TNF naïve patients and 49% of anti-TNF exposed patients were in remission at week 44, as compared with 36% of those receiving placebo. Ustekinumab was approved for treatment of moderate to severe CD in 2016.

A trial with identical doses was conducted for moderate to severe UC and published in 2019.(62) Five hundred and twenty-three patients who had failed conventional and prior biologic therapy were recruited and randomised to receive either an induction dose of 130mg IV, 6mg/kg IV, or placebo. Sixteen percent of patients receiving either 130mg or 6mg/kg achieved week 8 remission compared with 5% of placebo recipients. Among those who responded to ustekinumab induction, week 44 remission was significantly higher in patients receiving maintenance therapy (90mg every 8 weeks) compared to placebo.(62) Ustekinumab was approved for treatment of UC by the EMA in 2020.

#### *1.4.6.3 Risankizumab*

Risankizumab is a selective IL-23 inhibitor developed for treatment of psoriasis(207, 208) and psoriatic arthritis.(209). Unlike ustekinumab that targets the p40 subunit present in both IL-12 and IL-23, Risankizumab is a humanised monoclonal antibody targeting the p19 subunit that is exclusive

to IL-23. In the ADVANCED and MOTIVATE trials(210) patients who had not previously failed biologic therapy (ADVANCE trial) and patients who had loss of response to previous biologic therapy (MOTIVATE trial) were studied. In ADVANCE the primary end points were week 12 clinical remission and endoscopic response. Of 931 recruited patients, 373, 372 and 186 were randomised to IV risankizumab 600 mg, 1200 mg, or placebo respectively. In MOTIVATE, 618 were recruited with 206, 205 and 207 similarly randomised. In the ADVANCE trial, remission rates were significantly higher in both treatment arms with clinical remission and endoscopic response rates for 600mg, 1200mg, and placebo, being 45%, 43%, 25% and 40%, 32%, 12% respectively. In MOTIVATE findings were similar with clinical remission in 35%, 40%, and 19% and endoscopic response in 29%, 34% and 11% for risankizumab 600 mg, 1200 mg, and placebo respectively. Patients who achieved a response in either trial were recruited to the FORTIFY trial.(211) This 52 week trial included 542 patients randomised to either 180mg (179 patients) or 360mg (179 patients) risankizumab, or placebo (184 patients) given subcutaneously every eight weeks. Week 52 clinical and endoscopic remission were the co-primary endpoints. To account for different regulatory requirements between the FDA and EMA two definitions of clinical remission were used. A Crohn's Disease Activity Index (CDAI) <150 was required by the FDA while a stool frequency and abdominal pain score was required for EMA. Significantly more patients were in CDAI remission at week 52 with both risankizumab 180mg (55%) and 360mg (52.5%) compared with placebo (41%),  $p \leq 0.005$ . Based on abdominal pain and frequency while significantly more patients receiving 360mg (52%) were in remission compared with placebo (40%)  $p = 0.004$ , the remission rate in the risankizumab 180mg arm was not significantly higher than placebo ( $p = 0.12$ ). The endoscopic remission rate was the same and higher than placebo for both dose levels (47% vs. 22%,  $p = 0.001$ ).

#### 1.4.6.4 Combination therapy

Anti-drug antibody development is considered a significant mechanism underlying secondary treatment failure (i.e. loss of response following an initial good response). Combination therapy, as shown with IFX, can be advantageous in preventing antibody development.

The SONIC study, a double blinded RCT compared AZA or IFX monotherapy with IFX and AZA as combination therapy for moderate to severe CD. Patients receiving both drugs were more likely to be in steroid-free remission at week 26 (57%) than with either IFX (44%) or AZA (30%) alone and with no increase in serious infections.(212)

A similar study showed that the combination was more effective than monotherapy for patients with moderate to severe UC. The week 16 steroid-free remission rate was 40% in combination therapy compared with 22% and 24% for IFX and AZA monotherapy respectively.(213)

The reason for this synergistic effect is thought to relate to the lower levels of anti-drug antibodies in patients on combination therapy that includes an immunomodulator; 1% compared with 15% of patients on IFX monotherapy.(212)

A synergistic benefit of combination therapy was not found for ADA. The DIAMOND study included 176 patients with CD who were randomised to either monotherapy or combination therapy using ADA and AZA. In this open labelled prospective trial there was no difference in the primary end point of week 26 remission.(214) Anti-drug antibodies were assessed in 76 monotherapy and 75 combination therapy patients. Although there were numerically more patients with antidrug antibodies in those receiving monotherapy compared to combination therapy, the difference was not significant (10/76 vs. 3/75,  $p=0.078$ ). In a post hoc analysis, anti-drug antibodies were significantly associated with lower drug trough levels at week 52.(215)

Randomised control trial data for combination therapy is lacking for UC.(216) Given that ADA is a fully humanised antibody it is less likely to be immunogenic than IFX, a chimera with a mouse protein component. Thus, a lesser benefit from adding AZA to ADA might be anticipated.

Combination therapy is associated with an increased risk of infection. In a systematic review and meta-analysis that evaluated the risk of serious infections with combination therapy in IBD found that combinations that include TNF $\alpha$  antagonists, especially with corticosteroids, are associated

with higher risk of serious infection and that monotherapy with an immunosuppressive agent is associated with a lower risk of infection than monotherapy with a TNF $\alpha$  antagonist.(217)

#### *1.4.6.5 Drug Monitoring during Biologic Therapy*

A significant proportion of IBD patients fail biologic therapy. Failure can occur in the induction phase where patients never respond to therapy, i.e., primary non-response and occurs in up to a third of patients. Secondary loss of response occurs in 20–40% of patients who initially respond to treatment. One potential cause of secondary loss of response is the presence of inadequate or sub-therapeutic drug levels. Sub-therapeutic levels may result from loss of drug due in severe disease to a protein losing colopathy. Other postulated mechanisms to account for the sub-therapeutic drug levels include that the increased inflammatory burden upregulates production of the ligand specific to the monoclonal antibody, which binding to it effectively neutralise it, or development of anti-drug antibodies. Any of these mechanisms could lead to a reduction in the monoclonal antibody concentrations below the therapeutic level and allow disease reactivation.

Infliximab studies show that patients with a higher serum trough drug level (drug level taken immediately prior to infusion) are more likely to have a clinical and endoscopic response and remission. A recent multi-centred prospective study of multiple Predictors of failure of Anti-TNF therapy Study (PANTS) in luminal CD included 955 IFX and 655 ADA recipients. The study considered age, BMI, sex, smoking status, and history of perianal disease. Following multivariate analysis the only factor associated with remission at weeks 14 and 54 was the week 14 drug level.(218) Low serum trough levels were associated with increased IFX immunogenicity and development of anti-drug antibodies. Low ADA levels have also been associated with lower rates of endoscopic remission in luminal CD.(214)

#### *1.4.6.6 Dose optimisation strategy*

As low drug levels are associated with anti-TNF treatment failure, there is now some evidence to support dose optimisation strategies. By increasing the dose or decreasing the dosing Interval, the time that serum drug levels fall below therapeutic concentrations can be reduced. There are two

schools of thought regarding best strategy for dose optimisation; therapeutic drug monitoring with either prospective or reactive treatment strategies.(219) Prospective drug optimisation, as exemplified in the SERENE trial involves incorporating routine monitoring and dose adjustment according to the drug level as an inherent part of the treatment strategy.(220, 221) In reactive dose optimisation, drug levels are obtained only in response to patient's clinical symptoms; should a patient be significantly symptomatic, drug levels can be taken. If a patient has low drug levels or presence of antidrug antibodies is detected the dose can be optimised, by either decreasing the dosing interval or increasing the dose.(219)

There are no RCT of accelerated IFX dosing for acute severe UC. In the settings of steroid refractory acute severe UC, dose acceleration as a rescue therapy can be undertaken without waiting for drug levels.(222) A recent meta-analysis of open label cohort studies found no conclusive evidence to support an accelerated induction regimen.(223) It is however thought that clinicians were already incorporating biomarker results e.g., C-reactive protein (CRP) and albumin to guide clinical decision making which may have confounded the result. To clarify this, a propensity-matched cohort study of 102 patients with steroid-refractory acute colitis receiving standard induction compared to 29 with accelerated induction was conducted. This showed a decrease in the 30 day colectomy rate when matched for CRP, serum albumin, CRP/albumin ratio, haemoglobin and the presence of pancolitis (57% vs 27%,  $P=0.048$ ). For those completing induction therapy there was no subsequent decrease in the colectomy rate during the follow-up period (57% vs 31%,  $p=0.09$ ). (224) The TITRATE trial for acute severe UC is currently seeking to resolve the issue. It aims to compare whether a personalised dosing model for acute severe UC, based on demographic parameters (gender, body weight), blood chemistry (CRP, albumin), anti-drug antibodies, and trough levels, is superior to a standard dosing regimen. This may help to define the optimal IFX induction dosing regimen for acute severe UC.(225)

SERENE UC and SERENE CD are two recent RCTs investigating the effect of intensified dosing regimens for ADA induction in moderate to severe UC and CD.(220, 221) An intensified dosing

regimen of 160mg ADA at week 0, 1, and 3 was compared in a double blinded fashion with the standard dosing regimen of 160mg at week 0 and 80mg at week 2. For UC, week 8 remission rates were not significantly greater in the intensified regimen, 13% vs 10%. Similarly for CD, week 4 remission and week 12 endoscopic response rates were not significantly greater in the intensive dosing regimen 44% vs 44% and 43% vs 39% respectively.

Strategies of dose optimisation for patients with secondary ADA treatment failure include dose escalation to 80mg every two weeks or decreasing dosing interval from biweekly to weekly; both approaches are authorised by the EMA. The open label extension of the CHARM study of ADA in CD switched patients who lost response from biweekly to weekly therapy.(226) Of 71 patients changed to weekly therapy, 37% achieved clinical remission at week 54.

The CALM study was an open labelled RCT of ADA dose escalation based on CRP, faecal calprotectin and disease activity in CD.(227) Two hundred and forty four patients with active endoscopic CD were recruited and randomised to either tight control or clinical management arms. In the tight control group, patients were dose escalated based on CRP >5mg/L, faecal calprotectin >250µg (measured at weeks 11, 23, and 35), Crohn's Disease Activity index (CDAI) >150, or prednisolone use the previous week. Treatment escalation was allowed in the clinical arm based on CDAI (>200, or a decrease <100 compared with baseline) and prednisolone use. The primary endpoint was week 48 endoscopic remission (Crohn's diseases endoscopic index of severity (CDEIS) <4 and no large ulcers) This study showed that dose escalation based on close monitoring of biomarkers can lead to significantly greater endoscopic remission at week 48 for those with tight control compared to those with clinical management only, 56/122(46%) and 37/122(30%) respectively, p=0.010,. This study did not however incorporate drug monitoring.

TAILORIX(228) and TAXIT(229) are two prospective clinical trials which evaluated proactive therapeutic drug monitoring for IFX. TAXIT study recruited 263 IBD patients (178 CD, 85 UC) stable on maintenance IFX therapy that were randomised either to drug dose optimisation based on either proactive drug levels or clinical features. In the proactive drug level monitoring arm, doses were

adjusted aiming for a trough IFX concentration of 3–7µg/mL. In the proactive arm 69% were in remission at week 54 which did not differ significantly from the 66% remission rate in those dosed based on clinical features. In the TAXIT trial there were however fewer flares during maintenance in the proactive drug monitoring arm. Aiming for a maintenance trough level of 3–7µg/mL, 9/128 (7%) patients in proactive dosing arm required rescue therapy, compared with 21/123 (17%) patients with clinically based dosing,  $p=0.018$ .

TAILORIX recruited CD patients naïve to biologics to receive combination IFX and AZA therapy. After induction at week 14, patients were randomised to one of three IFX maintenance strategies. IFX was given every eight weeks from week 14 to week 54. In group 1 (45 patients) the IFX dose was increased by 2.5mg/kg increments to a maximum of 10mg/kg based on clinical symptoms, biomarkers and/or serum IFX trough level. Group 2 patients (N37) received a single incremental dose of 5mg/kg based on the same criteria and for the control group (N 40) the dose was increased by 5 to 10 mg/kg based on clinical symptoms alone. Serum IFX levels were measured prior to infusions during the induction phase and then every four weeks thereafter. The aim in the intervention arms was to achieve trough IFX levels of >3µg/mL. There was no significant difference in the primary endpoint, steroid free remission from week 22 to week 54 and endoscopic remission at week 54, between the groups.(228)

In both the TAILORIX and TAXIT trial the target IFX trough level was >3µg/mL, however in the PANTS study a serum concentration of >7µg/mL was required for association with week 14 and week 55 remission.(218) Neither trial considered other factors that could be associated with increased IFX clearance e.g., presence of anti-drug antibodies, CRP, serum albumin and body weight. In TAXIT, the IFX dose was reduced for patients with levels >7µg/mL. As more recent data analysis indicates that higher IFX concentration are beneficial in fistulising CD, the target level of 3µg/mL in these trials could have been too low and is a potential limitation of these studies.(230) Major strengths were that TAILORIX and TAXIT were well designed double blind RCTs with TAILORIX having 27 study sites across Europe with very complete follow up.

The PRECISION trial published in 2021 compared standard IFX dosing with an integrated model based dosing.(231) The model based dosing was calculated using IFX serum concentrations, anti-drug antibody levels, serum CRP and albumin (all measured at the mid-point between, and immediately pre infusions) together with patient-specific variables such as body weight and gender. The model aimed for a trough level of  $>3\mu\text{g/mL}$ . Sixty six patients with CD and 14 with UC in remission and stable on maintenance IFX therapy were enrolled with 40 patients randomised to each group. After one year, 28/32(88%) in the model based dosing arm remained in sustained clinical remission which was significantly higher than the 25/39(64%) of patients in the standard dosing arm,  $p=0.017$ . No difference in CRP levels were found however those with the proactive model based dosing had lower levels of faecal calprotectin after one year, ( $p=0.031$ ).

Higher serum trough levels of ADA have also been associated with clinical remission and endoscopic remission in both UC and CD.(218, 232, 233) The SERENE UC and SERENE CD trials did not show any advantage to drug level monitoring during induction of remission for either UC or CD. Each trial also examined the effect of therapeutic drug monitoring on maintenance ADA therapy. In SERENE UC patients who responded to ADA induction were randomised to receive maintenance therapy of 40mg of ADA either every week, every other week, or therapeutic drug monitoring (TDM).(220) The TDM arm aimed for a trough concentration of  $10\text{--}20\mu\text{g/mL}$  with doses escalated if trough levels were  $<10\mu\text{g/mL}$ , or if there was rectal bleeding present at a dose of between  $10\text{--}20\mu\text{g/mL}$ . A Japanese cohort of patients who were included in this study were unfortunately not randomised to the TDM arm. In the integrated study, that included the Japanese cohort, week 52 remission was higher in patients who received weekly compared with biweekly treatment, 72/175 (40%) compared with 49/163 (29%),  $p=0.045$ . However, those who had TDM were not significantly more likely to be in remission at week 52 with just 27/74 (36.5%) in remission.

The SERENE CD trial compared dose escalation during maintenance based on either TDM or clinical parameters. In the TDM arm the target was a minimum ADA concentration of  $5\mu\text{g/mL}$ . The remission rates at week 56 were similar irrespective of the strategy used. This lack of significance

was also apparent in the analysis of the secondary endpoints of week 46 steroid free remission, endoscopic response, endoscopic remission, and deep remission.(221)

When interpreting the results of therapeutic monitoring of biologics and comparing results across studies consideration should be given to the type of assays used and inter assay variability. Enzyme linked immunosorbent assays (ELISAs) are widely used however they are time consuming involving multiple wash steps. With ELISAs the presence of circulating drug interferes with the detection of antidrug antibodies.(234) Homogenous model shift assays (HMSA) use high performance liquid chromatography to quantify drug level based on the changes in molecular weight that occur in an antibody-antigen reaction.(235) This method allows for quantification of the drug and of anti-drug antibodies. Inter assay variability studies have shown that although there is correlation between assays, HSMA can yield a higher result than the corresponding ELISA test.(236) As yet there is no accepted gold standard test for assay of biologic agents. The TAXIT and TAILORIX trials used in house ELISA assays(228, 229). Care should thus be taken when interpreting results and comparing them across studies or to results in local laboratories.

#### *1.4.6.7. Novel Oral Agents*

##### *1.4.6.7.1 JAK STAT Pathway*

Janus Kinases (JAK) are members of the tyrosine kinase family that interact with the family of DNA binding proteins called the Signal Transducer and Activators of Transcriptions (STAT). JAK inhibitors are small molecule inhibitors that unlike biologic therapies can be taken orally and do not trigger development of antidrug antibodies. The activation of STAT proteins through phosphorylation by JAK proteins leads to regulation of multiple immune mediators including IFN- $\gamma$ , ILs-2, -3, -4, -7, -8, -12, -15, -21 and -23. Over 50 ligands have receptors that share JAK-STAT signalling pathways to mediate their effect including but not limited to ILs -2, -12, -23, INF- $\gamma$ , Oncostatin M, Leukaemia Inhibitory Factor, and Granulocyte Macrophage Colony Stimulating Factor (GM-CSF). The JAK family include four members, JAK1, JAK2, JAK3, and Tyrosine Kinase 2 (TYK2), while there are seven members of the STAT family, STAT 1, 2, 3, 4, 5A, 5B, and 6. Although other members of the JAK family are present in all cells, JAK3 is the most specific and is present only in haematopoietic cells.

Over time, tyrosine kinase inhibitors were developed targeting key pathways in disease pathogenesis. Imatinib for leukaemia treatment is based on inhibition of the BCR-ABL tyrosine kinase. Ruxolitinib, the first FDA approved JAK-STAT inhibitor, is selective for JAK1 and JAK3 and is approved for treatment of myelofibrosis, and multiple myeloma.(237).

#### 1.4.6.7.2. Tofacitinib

Tofacitinib was initially developed as a JAK3 inhibitor for use in immunosuppression for transplantation. However it became clear that tofacitinib also inhibited JAK1 and JAK2 although it preferentially inhibits JAK1 and 3. This drug limits differentiation of CD4 T helper cells and limits Th17 cell production. It was soon trialled and found to be effective for treatment of RA.(238, 239) As tofacitinib is a small molecule inhibitor it does not have the instability issues associated with biologic therapy and hence can be given orally. Non inferiority trials in RA showed that it was similar to ADA for treatment of RA and did not require parenteral administration.(240, 241) Following success in RA treatment, it was trialled for treatment of moderate to severe UC with two induction trials and subsequent maintenance studies; OCTAVE induction-1, OCTAVE induction-2, and OCTAVE sustain.(242) Patients in the induction study were randomised to receive either 10mg or 15mg of tofacitinib or placebo orally, twice daily for eight weeks. Six hundred and fourteen patients were recruited to OCTAVE induction-1; 122 received placebo, 16 patient received 15mg tofacitinib twice daily, however this dosage was abandoned early based on feedback from the regulatory authorities, and 429 patients received 10mg twice daily. Week 8 remission was present in 88/476 (18.5%) of those receiving 10mg tofacitinib compared with 10/122(8.2%) of placebo recipients,  $p=0.007$ . A total of 547 patients were recruited to OCTAVE induction 2. Remission at week 8 was present in 71/429 (16.6%) of the patients in the 10mg tofacitinib group and 4/112(3.6%) in the placebo group,  $p<0.001$ . Clinical responders in OCTAVE induction 1 and 2 were recruited to OCTAVE sustain. Five hundred and ninety three patients were randomised to receive either tofacitinib 5mg, 10mg, or placebo twice daily. Week 52 remission rates were significantly higher in both the 5mg and 10mg groups compared with placebo; 68/198 (34.3%), 80/197 (40.6%) and 22/198(11.1%) respectively.

Tofacitinib trials in CD have not been as promising as the UC studies. An Induction study recruited 139 patients to receive either 1mg, 5mg, or 15mg orally, twice daily of tofacitinib or placebo. This showed that week 4 response and remissions were not significantly higher in the treatment arms when compared with placebo.(243) In a subsequent RCT 280 patients were recruited to the induction phase of the study and received tofacitinib 5mg (86 Patients); 10mg (86 patients), 15mg (16 patients) or placebo (92 patients).(224) Week 8 remission rates were not significantly greater in the treatment arms compared to placebo. Overall, 180 patients achieved clinical response at week 8 and were recruited to the maintenance study. Patients were randomised to twice daily tofacitinib 5mg (n=43); 10 mg (n=43); or placebo (n=42). Week 26 remission rates were 37% , 42% and 29% for the 5mg, 10mg and placebo arms respectively. Although week 26 CRP levels did drop significantly in patients receiving 10mg twice daily when compared to placebo, there was no significant difference in 26 week remission between treatment arms.(244) Tofacitinib has not been authorised for CD.

In an open label study of patients with RA comparing tofacitinib with anti-TNF therapy, an increased risk of venous thromboembolic (VTE) disease and increased mortality was reported in a subgroup of tofacitinib recipients. These patients were aged 50 years or more and had at least one cardiovascular risk factor.(245, 246) The risk appears to be dose dependant with higher dose recipients were most affected. The European Medicines Agency (EMA) recommends against using tofacitinib 10mg twice daily as maintenance for UC and for it to be used with caution in patients with any risk factors for VTE.(247) The FDA has placed a black box warning on all JAK inhibitors.(248)

Tofacitinib is also associated with an increased risk of herpes zoster. In a pooled analysis of the aforementioned UC trials and open label extension studies an increased risk of herpes zoster was seen. (249) As for VTE, this effect appears to be dose dependent, with patients receiving 10mg twice daily having twice the risk of developing shingles as patients on 5mg twice daily or placebo.(250) Since 2017, a non-live vaccine is available that effectively prevents herpes zoster and may be given to those on immunosuppressive therapy.(251)

#### 1.4.6.7.3 Upadacitinib

Upadacitinib is another JAK/STAT treatment that has recently been approved for treatment of UC.

It was developed to selectively inhibit JAK1. It has a 40 fold greater affinity for JAK1 compared with JAK2 and over 100 times that for JAK3 or TYK2. Like previous treatments for IBD, it was developed initially as a treatment for rheumatological conditions. It was approved for psoriatic arthritis, RA, and atopic dermatitis before being investigated for UC.

Two separate UC upadacitinib induction trials, U-ACHIEVE and U-ACCOMPLISH were conducted. In U-ACCOMPLISH an induction dose of 45mg resulted in a week 8 remission rate of 26% compared with 5% for placebo. In U-ACHIEVE the rates were 35% and 4% respectively. Patients who responded to induction were enrolled in the maintenance trial, the U-ACHIEVE maintenance study. Daily doses of 15mg and 30mg were more effective than placebo with remission rates of 42%, 52% and 12% respectively at weeks 52. (252)

Unlike tofacitinib, a pan JAK inhibitor, upadacitinib is effective in treatment of CD. In the phase III study, U-EXCEED, 39% of patients who received upadacitinib 45mg/day for induction achieved week 12 remission compared to 21% of patients receiving placebo.(253) The U-EXCEL study reported a 49% week 12 remission rate compared to 29% for placebo.(253) Responders in these two studies were enrolled in the U-ENDURE maintenance study and randomised to receive either upadacitinib at 15mg, 30mg, or placebo. Week 52 clinical remission rates for 15mg (37%) and 30mg (48%) were significantly higher than placebo (15%). Endoscopic remission rates were similarly significantly better for 15mg (19%) and 30mg (29%) than placebo (5%).(253)

#### 1.4.6.7.4 Filgotinib

Filgotinib is another preferential JAK1 inhibitor that can be dosed daily. It is also effective for treatment of RA,(254) psoriatic arthritis,(255) and ankylosing spondylitis.(256) A large trial of filgotinib for moderate to severe colitis involved two induction studies and one maintenance study. In induction patients received filgotinib 100 mg/day (n=277), 200 mg/day (n=245), or placebo (n=137).(257) The week 10 remission rate was significantly higher with 200mg, but not with 100mg,

compared to placebo. Following induction placebo recipients continued to receive placebo and filgotinib recipients were randomised to either continue their current dose or receive placebo. At week 58, filgotinib 200mg recipients had a significantly higher remission rate (37%) than those in the respective placebo arm.(11%) Following this trial a dose of 200g was authorised for treatment of moderate to severe UC.

Filgotinib initially showed promise in treatment for CD. In the FITZROY study, a phase II RCT, week 10 clinical remission was significantly higher in patients receiving 200mg daily (47%) compared with placebo.(23%) However this effect was not present for the secondary endpoints of endoscopic remission and clinical response.(258) DIVERSITY, the phase III induction and maintenance study was completed in November 2022 and the final results are awaited however it is reported that the induction cohort failed to meet the primary endpoint and approval for treatment of CD will not be sought.(259)

#### 1.4.6.7.5 Sphingosine-1 phosphate (S1P) receptor modulators

Although effective for treatment of IBD, the safety profile of JAK inhibitors is of concern. The five sphingosine-1 phosphate receptor modulators (S1P<sub>1-5</sub>) are a new class of small molecule inhibitor that may be useful in IBD treatment. Sphingosine-1-phosphate (S1P) is a signalling lipid that is an important regulator of inflammation. S1P is excreted into the extracellular space by metabolically active cells. It is at its highest concentration in blood and lymphatic tissues due to the lower concentrations of the S1P degrading enzymes present. Conversely, the concentration of S1P is lower intracellularly and in interstitial spaces due to the presence of higher concentrations of the degrading enzymes. This concentration gradient is thought to be important in lymphocyte trafficking, encouraging movement away from immune organs such as the thymus and spleen and into the blood. S1P binds S1P receptors which are a class of five membrane derived receptors, S1P<sub>1-5</sub>. S1P<sub>1-3</sub> are widely expressed throughout the body while S1P<sub>4</sub> and S1P<sub>5</sub> are expressed on lymphoid, haematopoietic, and central nervous tissue. S1P production is triggered by pro inflammatory cytokines such as TNF- $\alpha$ , VEGF, and IL-1.(260) The increased S1P concentration at

sites of inflammation allows lymphocytes expressing S1P<sub>1</sub> to migrate to the site of inflammation from lymphatic tissue. Fingolimod, the first S1P modulator, was derived from a mushroom used in traditional Chinese herbal medicine.(261) It antagonises all S1P receptors and is an effective treatment for multiple sclerosis.(262) Fingolimod has been associated with significant but rare side effects such as bradycardia, heart block, and macular oedema,(263) that are attributed to its non-selective S1P modulator activity as S1P<sub>3</sub> can be found in cardiac tissue.(264) Ozanimod was developed as a selective S1P modulator for the receptors S1P<sub>1</sub> and S1P<sub>5</sub>. Following successful phase I and II trials, the True North phase III trial assessed ozanimod as induction and maintenance treatment for UC.(265) Week 10 remission was significantly more common in patients receiving 1mg ozanimod compared to placebo (18% vs 6%), p<0.001 In the maintenance study, patients receiving 1mg ozanimod daily were significantly more likely to be in remission at week 52 than with placebo (37% vs. 18.5% respectively, p<0.001).(265) Phase III trials with ozanimod for induction and maintenance treatment of CD are underway with results anticipated in 2024.(266)

#### 1.4.6.7.6 Advantages of Novel Oral Agents

The novel oral agents have significant advantages over biologic therapy for IBD. They do not require clinic attendance for infusion or training for self-injection, which some patients may find undesirable. With these drugs there is less inter-patient pharmacokinetic variability, they lack the immunogenicity associated with biologic therapy and have shorter half-lives.(267) This can be a benefit as it allows faster recovery from immunosuppression when needed e.g., pre-operatively or in cases of infection. On the other hand, there is less data regarding their use in pregnancy and in animal studies, at higher doses than clinically used, they have been linked with fetal harm. Thus they are not recommended for use during pregnancy or conception.(268)

#### 1.5. Biomarkers of IBD Therapy Outcomes

A number of clinical, biochemical, endoscopic and immunological factors have been associated with a lack of response to biologic therapy in IBD among which are longer disease duration, level of clinical and endoscopic disease activity, faecal drug loss, previous anti-TNF exposure, increased CRP, decreased serum albumin, and increased tissue TNF- $\alpha$  expression.

### 1.5.1 C-Reactive Protein

C-reactive protein (CRP) was originally discovered in the 1930s by Tillet and Francis during investigation of serum samples obtained from patients with pneumococcal infection. It is a homo pentameric protein that can increase up to 1,000 fold at sites of inflammation. It is synthesised primarily in the liver in response to cytokines including IL-1 and IL-6 and is easily detectable in the serum. Due to its ease of detection and its short half-life it has become one of the most common clinically used markers of active inflammation.(269)

The data regarding CRP's utility as a marker of IBD disease activity are not consistent. In paediatric patients a CRP of  $\leq 3\text{mg/L}$  is present at diagnosis in up to 28% patients with CD and 52% of those with UC.(270) In adults with IBD, although CRP is not disease specific it is widely used as a marker of disease activity and correlates well with endoscopic, radiological, and clinical disease activity.(271-273) Prior to diagnosis, adult patients with gastrointestinal symptoms and a CRP  $< 5\text{mg/L}$  have a less than 1% probability of having IBD.(274) However, CRP is less effective in determining disease activity in those already known to have IBD. In a pooled analysis of over 19 studies an elevated CRP had a sensitivity of 92% in identifying those with endoscopically active IBD however the specificity was low at just 49%.(275)

There are also conflicting data regarding the CRP's utility as a marker of response to biologic therapy. A higher baseline CRP has been shown to be predictive of anti-TNF treatment failure in patients with UC refractory to steroids.(186, 204, 276-278) Yet there is some evidence that a higher CRP ( $> 3\text{mg/L}$ ) may be a predictor of UC response to IFX.(279)

In CD the data is equally mixed. Studies have shown that higher baseline CRP levels are associated with IFX response.(280, 281) These findings appear consistent with the post hoc analysis of the CHARM study for ADA. This showed that those with a baseline CRP greater  $\geq 10\text{mg/L}$  were twice as likely to be in remission at week 54.(282) Conversely, a Korean cohort study of 250 patients with CD undergoing ADA induction reported that an elevated CRP was an indicator of poor treatment response.(283) In a post-hoc analysis of the VDZ GEMINI study, a lower baseline CRP associated

with treatment response in CD.(284) This is consistent with reports from other retrospective observational studies indicating that a higher CRP is more likely associated with treatment failure.(285, 286)

The apparently conflicting results regarding CRP as a predictor of disease activity may be the result of confounding due to the nonspecific nature of CRP. It is elevated in a variety of conditions unrelated to IBD including, infection, immune mediated diseases, obesity, neoplasia, and trauma. Significant CD symptoms in an individual with a normal CRP can be indicative of fibro-stenotic disease which is refractory medical therapy and is better managed with endoscopic dilation, diet modification, or surgery. Thus, while CRP can be a useful indicator of disease burden it should not be relied upon in isolation and should be interpreted in the overall clinical context.

#### 1.5.2 Serum Albumin

Low serum albumin levels have been used as a biomarker of disease severity, this is thought to be due to a protein losing enteropathy that can be present in severe IBD.(287) Low serum albumin is linked poor outcomes in patients presenting with acute colitis.(288) Lower albumin is also associated with lower trough drug levels across a range of monoclonal antibodies used to treat oncological conditions,(289) and with IFX in treatment of IBD.(290, 291) This may be because a low albumin is a marker of disease activity and may reflect increased drug metabolism.

#### 1.5.3 Faecal Calprotectin

Faecal Calprotectin is a protein found abundantly in neutrophils and to a lesser extent in monocytes and macrophages. It induces cell receptor expression and is involved in cellular migration, adhesion, and phagocytosis. It is considered an acute phase protein and a marker of inflammation. It is a very stable protein resistant to degradation by bacterial or pancreatic enzymes and thus has been found to be a useful marker of inflammation in the gut. It is used to help differentiate between symptoms related to irritable bowel syndrome and IBD. Several studies have examined the association of faecal calprotectin levels with endoscopic activity in both UC and CD and it has even been proposed as a surrogate marker of mucosal healing.(275). In anti-TNF naïve patients early faecal calprotectin

normalisation was associated with treatment response to IFX.(276) In that study of 53 patients undergoing IFX induction, those with endoscopic remission at week 10 had a more rapid and steeper decline in faecal calprotectin than those who did not achieve remission ( $p < 0.001$ ). An 80% decrease in faecal calprotectin by week 2 or a week two level of  $< 50 \mu\text{g/g}$  was predictive of week 10 remission.(292) In a separate study faecal calprotectin normalisation (defined by the authors as  $< 100 \mu\text{g/g}$ ) following induction was predictive of anti-TNF therapy response among patient with IBD.(293). However the predictive value of calprotectin obtained later in the treatment course is less clear. Of 50 patients in clinical remission at week 14 following IFX induction faecal calprotectin results were not predictive of relapse free remission out to a year for Crohn's patients.(294) Yet a recent meta-analysis of 24 prospective studies concluded that patients with IBD in remission with a faecal calprotectin level greater than  $152 \mu\text{g/g}$  have higher risk of relapse within 24 months.(295) A potential role for faecal calprotectin in monitoring UC treatment response has been proposed with values of  $< 50 \mu\text{g/g}$  in patients with symptom resolution ruling out severe endoscopic activity.(296)

#### 1.5.4 Oncostatin M

Oncostatin M (OSM) is a member of IL-6 cytokine family.(297) OSM expression is increased in inflamed intestinal tissue from patients with moderately active UC compared with healthy controls.(297, 298) In an analysis of 200 patients with IBD, including two cohorts from phase III clinical trials of IFX and golimumab, high pre-treatment tissue OSM expression was strongly associated with anti-TNF therapy failure.(298) This was not confirmed in a paediatric IBD cohort. There, OSM and OSM receptor mRNA were not higher in intestinal biopsies of paediatric IBD patient who responded to IFX compared to non-responders.(299)

In IBD, baseline serum OSM levels have been associated with treatment response to anti-TNF in some (300-304) but not in all studies (305, 306). The value of OSM as a predictor of treatment response may be drug specific, as lower serum baseline levels of OSM were not associated with week 54 remission in patients being treated with VDZ.(303, 306)

### 1.5.5 Tumour Necrosis Factor- $\alpha$

Tumour Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) is a cytokine protein initially identified as a serum factor that induced apoptosis in cancer cells.(161) Targeted therapy directed at TNF- $\alpha$  is now first line biologic therapy for moderate to severe IBD. In CD, intestinal expression of higher concentrations of TNF- $\alpha$  are associated with more severe disease(307) and anti-TNF treatment failure.(308) Serum TNF- $\alpha$  levels are associated with treatment failure in fistulising Crohn's disease(309) and mucosal expression of lower TNF- $\alpha$  mRNA is predictor of response to IFX therapy in UC.(310) Currently neither serum nor mucosal TNF- $\alpha$  is routinely used as a biomarker in IBD or marker of treatment failure.

### 1.5.6 Biomarkers Under Investigation

Research has sought to identify other possible markers of disease response. Soluble (s) MAdCAM- 1 has been shown to be a useful in predicting response to treatment. In a recent study of 62 patients with IBD (CD and UC), undetectable levels of sMAdCAM-1 before VDZ induction were positively predictive of clinical remission. In the same study, baseline retinoic acid concentrations  $<1.05\text{ng/mL}$  were also associated with clinical remission.(311) Retinoic acid upregulates  $\alpha_4\beta_7$  expression on lymphocytes. With higher concentrations of retinoic acid leading to increased  $\alpha_4\beta_7$ , expression a reduction of the effect of VDZ might be anticipated. Conversely in the absence of, or with lower levels of retinoic acid, the ability of VDZ to successfully block  $\alpha_4\beta_7$  could be more achievable.

In a prospective study of 28 patients receiving VDZ therapy, prospective serum samples were collected and 37 common inflammatory mediators were assessed by a multiplex ELISA.(312) High circulating levels of IL-6, an important cytokine in Th1 mediated inflammation, was identified as predictive of VDZ treatment failure. In the same study high osteocalcin levels were also associated IBD responsiveness to VDZ therapy. Osteocalcin, produced by osteoblasts, is a marker of bone formation. High levels may simply reflect the presence of less systemic disease activity and less disturbance of bone metabolism. Osteocalcin is not thought to exert any direct effect on the gut inflammatory process.

## 1.6 Vedolizumab

Vedolizumab (VDZ) is a relatively new treatment for IBD. It is a fully humanised monoclonal antibody that recognises and blocks the  $\alpha_4\beta_7$  integrin protein.(313, 314) Integrins are cell-surface proteins variably expressed on circulating B and T lymphocytes. They interact with adhesion molecules on the endothelial cells in the lumen of blood vessels. Unlike natalizumab which blocks the  $\alpha_4$  subunit and is used in the treatment of MS (315) VDZ inhibits only the  $\alpha_4\beta_7$  integrin that selectively binds to MAdCAM-1 on intestinal vasculature. By blocking the  $\alpha_4\beta_7$  integrin, VDZ selectively blocks gut lymphocyte trafficking, and exerts an anti-inflammatory effect on intestinal tissue. VDZ has been shown in a phase III RCT to be an effective treatment for UC and CD, however a significant proportion, up to 63%, of patients fail to respond to induction therapy and 58% fail to reach clinical remission at week 54.(313, 314, 316-318)

The concentration of anti-TNF monoclonal antibody during induction is important determinant of the outcome of induction therapy.(319, 320) However, the relationship between the monoclonal antibody concentration and therapeutic response depends not solely on their pharmacokinetics (Pk) but also on a variety of pharmacodynamic (PD) factors. PK-PD factors are interrelated and subject to a number of influences including the target antigen characteristics, patient, and disease related factors.(321) In a recent PK-PD evaluation of VDZ using data from phase I, II, and the GEMINI phase III, CD and UC clinical trials the half-life ( $T_{1/2}$ ) of VDZ was 25.5 days.(322) Inter-individual drug clearance variability was considered to be moderate-to-large and was unexplained. Only extreme albumin and body weight values were identified as potential clinically important predictors of drug clearance. Endoscopic Mayo sub-score exhibited a weaker effect on drug clearance. Concomitant immunomodulators did not influence drug clearance, nor did the presence of antibodies to VDZ. The evaluation was limited by the low incidence of anti-drug antibodies observed in the GEMINI trials.(313)

In population PK analyses of anti-TNF monoclonal antibodies an association between low serum albumin concentration and increased drug clearance has been reported although the mechanism of this interaction is incompletely understood.(290)

In UC, decreased serum albumin is a marker of inflammatory disease burden. Increased inflammatory burden may be associated with increased target antigen expression. It is plausible that decreased serum albumin concentration is associated with increased VDZ clearance because of increased antibody-antigen binding and consequent drug neutralisation.

Another possible explanation is that inflammation of the gastrointestinal tract results in the elimination of monoclonal antibodies via a protein-losing colopathy. Albumin may therefore serve as a surrogate marker for loss of endogenous IgG and monoclonal antibodies through the gut. This hypothesis is in part supported by observations that of IFX treated patients with severe colitis, high faecal concentrations of IFX were detectable in those failing therapy.(311)

Research into requirements for dose escalation identified the importance of week 6 VDZ levels in treatment outcome. High week 6 VDZ levels are associated with endoscopic remission in prospectively collected observational studies.(284, 323) Higher drug levels do not, however, lead to greater saturation of the  $\alpha_4\beta_7$  integrin.(324) This may indicate that low drug levels are a surrogate marker for disease activity rather than a direct cause of loss of response.

Vedolizumab represents an important addition to the therapeutic options for patients with IBD and offers benefit even to some who fail first line biologic therapy. However, as yet there is no baseline biomarker that clearly predicts VDZ treatment outcome. Further research into biomarkers of VDZ responsiveness is required.

## 1.7 This Thesis in Context

Despite the recent growth in novel and effective treatments for IBD, there remains a very significant patient cohort who fail treatment and for some others the complications of existing therapies lead to their cessation. At best, treatment success is limited, with more than 50% of patients failing to

achieve remission and up to 70% of patients requiring a change of therapy within five years.(2-5) With CD, long-term complications, fistulisation and stenosis occur in around a third of patients within five years and in up to 50% of patients within 20 years of diagnosis, significantly impacting their quality of life.(325) With UC, despite best therapies 10% will require colectomy within 10 years of diagnosis. While, there remains a significant unmet need for newer and better therapies(326) the ability to more precisely target those for whom the existing treatments will afford durable treatment response is also required.

Vedolizumab is part of the next generation monoclonal antibody therapies designed to avoid some of the complications of former therapies. It is less immunogenic and avoids systemic immunosuppression yet can achieve effective local control of gut inflammation. The individual response to treatment is variable and while some patients experience early treatment failure others have a sustained, relapse free, response. Research is needed to further elucidate the determinants of treatment success or failure

The hypotheses are (i) that there are predictive markers of VDZ treatment outcomes and (ii) that VDZ alters the inflammatory protein composition in serum and in the local tissue microenvironment. Detailing the changes brought about by VDZ and examining their association with treatment outcome could reveal patterns of response predictive of success i.e. identify a biomarker or biomarkers of treatment success or indeed of failure.

To test these hypotheses, a series of studies that in a stepwise progression examine the role of VDZ drug levels and VDZ's effect on inflammatory proteins in the serum and in the tissue microenvironment are undertaken aiming to identify biomarkers of treatment response.

- 1) The first study examined the utility of VDZ trough levels during induction and maintenance therapy of IBD patients to predict steroid-free remission and persistence on therapy. This included a cross-sectional snapshot study of patients established on VDZ maintenance therapy and a prospective study recruiting patients due to start VDZ therapy.

- 2) The second study assessed the impact of VDZ induction on a panel of serum inflammatory markers in patients with IBD to determine whether the baseline concentrations of any might be predictive of a therapeutic response at weeks 14 and 30. This prospective study recruited patient due to start VDZ therapy. Serum samples were obtained prior to infusion at week 0 and week 6 of induction with a median clinical follow of 17.8 months.
- 3) The third study uses the human explant model to assess the effect of VDZ on the secreted inflammatory proteins of the tissue microenvironment. Using colonic explants donated from UC patients undergoing routinely scheduled endoscopy the effect of VDZ incubation on the colonic tissue secretome and its interaction with peripheral blood mononuclear cells is examined.

The research presented in this thesis expands the knowledge base relating to the use of VDZ in IBD treatment and seeks to profile serum and tissue biomarkers of VDZ therapeutic response. The results presented represent some steps along the pathway toward achieving that aim and pave the way towards a more precise understanding of how to select patients most likely to benefit from VDZ and thus towards personalised drug treatment strategies.

## Chapter 2.0: Clinical Relevance of Trough Drug Levels Obtained During Maintenance and During Induction Therapy with Vedolizumab in IBD

## 2.1 Introduction

The  $\alpha_4\beta_7$  integrin, a cell-surface protein, is variably expressed on circulating B and T lymphocytes. It interacts with Mucosal vascular Addressin-Cell Adhesion Molecule-1 (MAdCAM-1) on the endothelial cells of the intestinal vasculature. This interaction enables lymphocyte migration to the gut. Vedolizumab (VDZ) is a humanised monoclonal antibody that specifically recognises and inhibits the  $\alpha_4\beta_7$  integrin. It selectively blocks gut lymphocyte trafficking and thus exhibits an anti-inflammatory effect on intestinal tissue. Originally delivered only intravenously (IV) by infusion, a subcutaneous formulation is now available.

In a phase III randomised controlled trial, VDZ was efficacious as an induction and maintenance agent for inflammatory bowel disease (IBD) including Crohn's disease (CD)(314) and ulcerative colitis (UC).(313) Realworld retrospective studies have also confirmed that it is an effective treatment.(327-331) Previous studies have shown an association between clinical and endoscopic remission in IBD patients receiving VDZ for treatment and their week 6 VDZ trough levels.(332, 333) Further characterisation of the pharmacokinetics of VDZ in IBD during the induction phase and during maintenance therapy is needed to add to the existing knowledge base.(334, 335). Here we report the results from two such studies; the first, the maintenance study, expanding the knowledge related to VDZ during maintenance and the second, the induction study, focusing on that during treatment initiation.

## 2.2 Specific Aims

In the maintenance study there were three specific aims:

1. Determine whether maintenance VDZ trough levels are associated with clinical disease activity
2. Evaluate the impact of prior anti-TNF therapy, concomitant azathioprine, or concomitant methotrexate therapies on VDZ trough concentrations.
3. Assess the association of VDZ trough levels obtained during maintenance with biochemical markers of disease activity

In this prospective induction study there were four specific aims:-

1. Determine whether VDZ trough levels obtained during induction at week 6 are associated with week 14 and week 30 steroid free remission (SFR)
2. Determine if there is a VDZ trough levels during induction that is predictive of SFR
3. Assess the associations of VDZ trough levels obtained during induction with VDZ treatment persistence.
4. Assess the associations between VDZ induction therapy and biomarkers of disease activity including serum C-reactive protein (CRP) and albumin, and faecal calprotectin

## 2.3 Methods

### 2.3.1 Study Population

This was a multi-centre prospective study of patients with IBD receiving VDZ therapy. Patients with an established diagnosis of CD or UC who were due to start or were already receiving VDZ were prospectively identified at three academic medical centres that are members of the Investigator Network for Inflammatory bowel disease Therapy in Ireland (INITIative). Patients were deemed ineligible if they were: aged less than 18 years; unable to give consent; had previously failed VDZ therapy; were pregnant; or breastfeeding. Informed consent was obtained from all participants at enrolment.

On enrolment, patients were assigned to the maintenance study, cohort A, or the induction study, cohort B, according to whether they were already established on VDZ therapy i.e., had received VDZ for at least 10 weeks (cohort A) or were due to start therapy (cohort B).

For all enrolled patients baseline demographic and clinical data including disease and medication history were obtained by patient questionnaire complemented by medical record review at the time of enrolment. Variables collected included: age, weight, gender, disease duration and activity score, 5-aminosalicylic acid (5-ASA) use, concurrent immunomodulator use (thiopurines/methotrexate), previous biologic therapy exposure (IFX, adalimumab, ustekinumab, golimumab), previous small molecule inhibitors (tofacitinib), and current corticosteroid therapy. Disease extent and location was classified using the Montreal classification.(336) Disease activity

was assessed using the Harvey Bradshaw Index (HBI) for CD,(337) and the partial Mayo subscore for UC.(338, 339) After enrolment the planned follow-up for all patients extended to at least one year post enrolment.

### 2.3.2 Ethics and Consent

This project was reviewed and approved by the research ethics committees of St James' and Tallaght University Hospitals (REC 2017–09); St Vincent's University Hospital (approved 18 September 2017); and Connolly and Beaumont Hospitals (reference number 17/49). Written informed consent was obtained prior to enrolling each patient.

### 2.3.3 Vedolizumab administration

#### 2.3.3.1 Maintenance study

In the maintenance study, patients were already established on VDZ having completed at least 10 weeks of therapy and, as directed by their treating physicians, were receiving VDZ every six or every eight weeks.

#### 2.3.3.2 Induction study

In the induction study VDZ was administered according to the standard protocol with all patients receiving 300mg infusions at weeks 0, 2 and 6. Patients with CD received an additional 300mg infusion at week 10. Following induction all patients continued on maintenance therapy with a VDZ infusion every six or every eight weeks as directed by the treating physician.

### 2.3.4 Sample Collection and Analysis

#### 2.3.4.1 Maintenance study

Serum samples for VDZ level and anti-VDZ antibody levels were obtained immediately prior to a scheduled infusion. Serum VDZ concentration and antibodies to VDZ were quantified using a commercial enzyme-linked immunosorbent assay (ELISA) (IDKmonitor® Vedolizumab drug level/Anti-Vedolizumab ELISA, Immunodiagnostic Stubenwald-Allee 8a, D-64625 Bensheim). Complete blood count, biochemistries (liver and renal profile), serum albumin and CRP were also measured at the same time points. When possible a stool sample for faecal calprotectin was also collected.

#### 2.3.4.2 Induction study

Serum samples for VDZ levels were obtained immediately prior to infusion and 30 minutes after infusion. Serum samples for quantification of antibodies to VDZ were obtained immediately before the fourth infusion. VDZ trough levels and antibodies to VDZ were measured as described above. Complete blood count, serum biochemistries (liver and renal profile), serum albumin and CRP were measured before each VDZ infusions at weeks 0, 2, and 6.

Faecal samples were obtained at baseline prior to treatment and within the 24 hours preceding each VDZ infusion. The faecal samples were collected by the patient into a standard faecal specimen container and refrigerated pending transport to the hospital. On receipt in the hospital they were dispatched to the hospital laboratory. Each sample was divided in two; with one part refrigerated (0–4°C), batched and tested for faecal calprotectin using a validated commercial laboratory assay. The second part of the sample was stored at -80°C for later measurement of VDZ levels.

To measure faecal VDZ, the protocol developed by Brandse et al. to measure IFX was followed.(311) Faecal samples were defrosted in 1:5 solution of Phosphate Buffered Saline (PBS) containing 6% Bovine Serum Albumin (BSA). Samples were vortexed for 60 minutes to homogenise and centrifuged at 3000g for five minutes. The supernatant was collected and the VDZ drug level determined in the same manner as for serum samples.

#### 2.3.5 Endpoints and Definitions

Crohn's disease activity was measured by the HBI. This is a validated scoring system developed by RF Harvey and JM Bradshaw in the 1980s. It assesses disease activity based on: number of liquid stools in 24 hours; abdominal pain; general well-being; presence of abdominal mass; and presence of extra intestinal manifestations.(337, 340) UC disease activity was measured by the partial Mayo sub score. This is a component of the Mayo subscore, a recognised and validated scoring system developed by Schroeder et al. It assesses disease activity based on stool frequency, rectal bleeding score, and physicians global assessment.(338, 339)

Disease activity was assessed at baseline, week 14, and week 30. SFR was defined as; a HBI of less than or equal to five for CD; a partial Mayo sub-score of less than or equal to one for UC; no requirement for glucocorticoid steroid therapy; and continuing treatment with VDZ. The Primary endpoint was week 14 SFR with week 30 SFR and VDZ treatment persistence as secondary endpoints.

#### 2.3.6 Statistical Analysis

Descriptive statistics were applied to the baseline demographics with continuous variables presented as the median and range and categorical variables summarised as percentages. The Mann Whitney U test was used for categorical and continuous independent data. Paired continuous data was interpreted using the Wilcoxon sign-rank test. Pearson's correlation was used to compare continuous variables.

Pre-specified analyses included the association of week 14 and week 30 SFR with baseline serum VDZ trough concentrations and the association of VDZ trough concentration with SFR and with the biomarker concentrations. Kaplan–Meier survival curves were constructed for time based analyses and differences between groups evaluated using the log-rank test.

All comparisons with a  $p \leq 0.05$  were considered significant. All statistical analyses were performed using SPSS (version 26.1.2; IBM, NY, USA). Graphs were constructed using GraphPad Prism (version 5.01 : GraphPad Software Inc.).

## 2.4 Results

### 2.4.1 Total Patient Population

A total of 78 patients were recruited to the study. Four patients in the induction cohort were excluded from analysis; one withdrew consent, one required colectomy within 48 hours, and two had no baseline samples. Seventy-four patients with IBD; 39 male and 35 female; 37 CD and 37 UC; 34 on maintenance therapy and 40 receiving induction therapy participated (**Figure 2.1**) The median age of 74 included patients at enrolment was 44.8 years [range 18.1–75.85 years]. The median disease duration was 11.5 years [range 0.3–45.14 years]

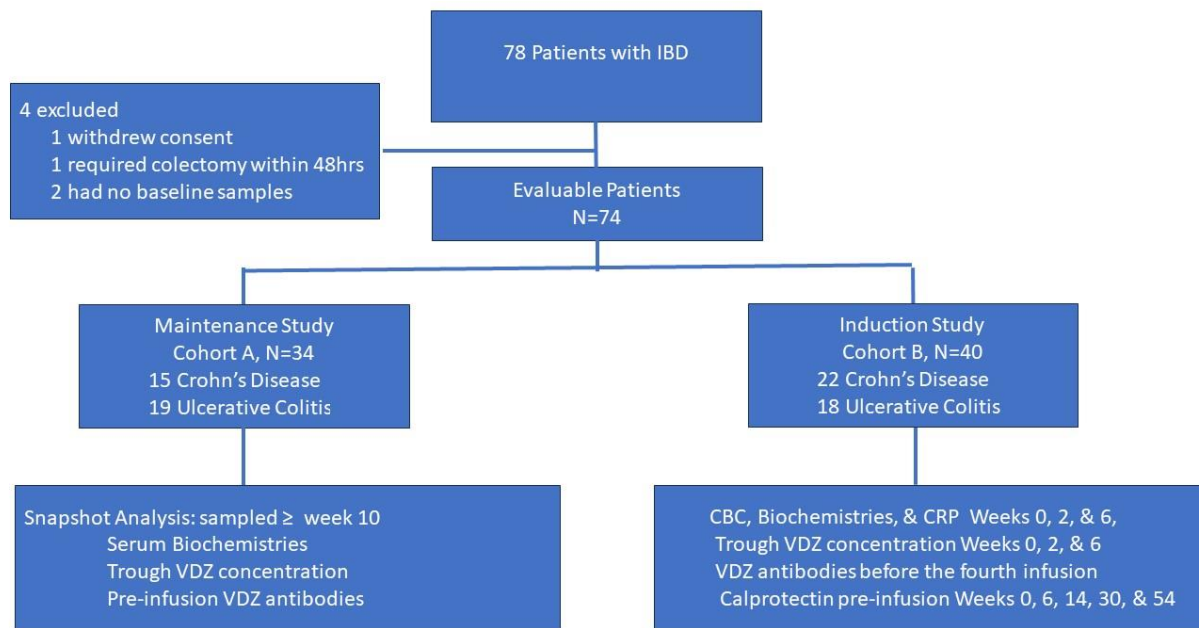


**Figure 2.1: Vedolizumab Trough Concentration Study Flow Diagram**

Seventy eight patients were recruited to the study. Four were excluded from analysis; one withdrew consent, one required colectomy within 48 hours, and two had no baseline samples. Seventy-four evaluable patients with IBD; 39 male and 35 female; 37 CD and 37 UC; 34 on maintenance therapy and 40 receiving induction therapy were included in the analyses.

## 2.4.2 Maintenance Study

### 2.4.2.1 Maintenance Study Patient Characteristics

**Table 2.1** summarises the demographics of 34 patients recruited to the study. Sixteen patients were recruited from St James Hospital Dublin, 11 patients from Connolly Hospital Blanchardstown, and seven from Beaumont Hospital. All patients were established on VDZ therapy for a minimum of 10 weeks and had completed their induction regimens. The mean duration of therapy prior to enrolment was 10.6 months [range 2.3–30.5 months]. Twenty-seven (79%) patients were established on standard dosing of VDZ with an infusion once every eight weeks. The remaining seven patients received an infusion once every six weeks. No patients was receiving concomitant biological therapy. Only one patient was on oral glucocorticoid steroids, however, four patients were receiving concomitant budesonide enemas. At time of sampling, fourteen patients (41%) were in SFR.

| <b>Cohort A: Vedolizumab Maintenance Study</b>                                                                                                                                                                                                                  |                                                                                                 |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|
|                                                                                                                                                                                                                                                                 | N=34                                                                                            |
| <b>Age (years)</b><br>Median [range]                                                                                                                                                                                                                            | 44.3 [17.7–76.2]                                                                                |
| <b>Gender</b><br>Female<br>Male                                                                                                                                                                                                                                 | 16 (46%)<br>18 (54%)                                                                            |
| <b>Disease duration at VDZ initiation (months)</b><br>Median [range]                                                                                                                                                                                            | 15.4 [1.3–45.1]                                                                                 |
| <b>Duration of VDZ Therapy (Months)</b><br>Median [range]                                                                                                                                                                                                       | 10.6 [2.3–30.5]                                                                                 |
| <b>Crohn's Disease (n=15)</b><br><b>Disease Extent</b><br>Ileal<br>Colonic<br>Ileocolonic<br>Isolated upper gastrointestinal tract<br><b>Disease behaviour</b><br>Non-stricturing non-penetrating<br>Stricturing<br>Penetrating<br><b>Harvey Bradshaw Index</b> | <br><br>1 (7%)<br>6 (40%)<br>6 (40%)<br>2 (13%)<br><br>7 (47%)<br>1 (7%)<br>7 (47%)<br>4 [0-13] |
| <b>Ulcerative colitis (n=19)</b><br><b>Disease Extent UC</b><br>Proctitis<br>Left-sided colitis<br>Extensive colitis<br><b>Partial Mayo Subscore</b>                                                                                                            | <br><br>2 (10%)<br>7 (37%)<br>10 (52%)<br>3 [0–8]                                               |
| <b>Laboratory parameters</b><br>Faecal calprotectin (µg/g) (n=13)<br>C-reactive protein (mg/L) (n=21)<br>Albumin (g/L) (n=21)                                                                                                                                   | <br>277.5 [24–>1000]<br>3.7 [0.3–28]<br>41 [35–48]                                              |
| <b>5-Aminosalicylates</b>                                                                                                                                                                                                                                       | 13 (37%)                                                                                        |
| <b>Oral Corticosteroids</b>                                                                                                                                                                                                                                     | 1 (3%)                                                                                          |
| <b>Immunomodulators</b><br>Thiopurines<br>Methotrexate                                                                                                                                                                                                          | 7 (20%)<br>3 (9%)<br>4 (11%)                                                                    |
| <b>Anti-TNF Therapy</b><br>Naïve<br>≥1 previous agent<br>≥2 previous agents<br>≥3 previous agents                                                                                                                                                               | <br>6 (18%)<br>28 (82%)<br>18 (52%)<br>3 (8%)                                                   |

**Table 2.1: Demographics and Clinical Characteristics of Maintenance Study Participants (n=34) at Enrolment**

\*Categorical variables presented as percentages

\*\*Continuous variables presented as mean [range]

#### 2.4.2.2 Maintenance Study: VDZ Trough Concentrations

The median [range] trough VDZ concentrations of all 34 maintenance patients was 9.5µg/mL [0–24.9µg/mL]. The median [range] trough concentrations for those with CD (n=15) was 8.1µg/mL [4.8–24.9µg/mL] and for those with UC (n=19) was 9.5µg/mL [0–16.6µg/mL] with no significant difference by disease diagnosis, p=0.33.

Similarly, no overall difference in the median trough VDZ concentrations between the 14 patients with SFR and the 20 without SFR was found with median [range] levels of 9.6µg/mL [3.4–24.9µg/mL] and 8.95µg/mL [0.0–19.2µg/mL] respectively (**Figure 2.2**).

Of 15 patients with CD, no difference in median [range] trough concentration was found by disease status, SFR (n=6) or active disease (n=9), at the time of sampling with median [range] levels at 8.4µg/mL [3.4–10.5µg/mL] and 9.6µg/mL [0.0–16.6 µg/mL] respectively (**Figure 3.2**).

However, for the 19 patients with UC, a numerically higher median [range] trough concentration was found for those in SFR (n=8), 11.2µg/mL [5.9–24.9µg/mL] compared with 6.9µg/mL [5.4–19.2µg/mL] for those with persistent disease activity (n=11), a difference that fell short of achieving statistical significance (**Figure 2.2**). Antibodies to VDZ were not detected in any of 34 samples tested.

In an opportunistic post-hoc analysis of the maintenance cohort wherein 27 patients received VDZ every eight weeks and seven patients were receiving infusions every six weeks no significant difference trough concentrations was detected the medians [ranges] were 9.96µg/mL [0 – 249µg/mL] and 10.26µg/mL [5.4 – 21.19µg/mL] for 8 weekly and 6 weekly infusions respectively, p=0.118.

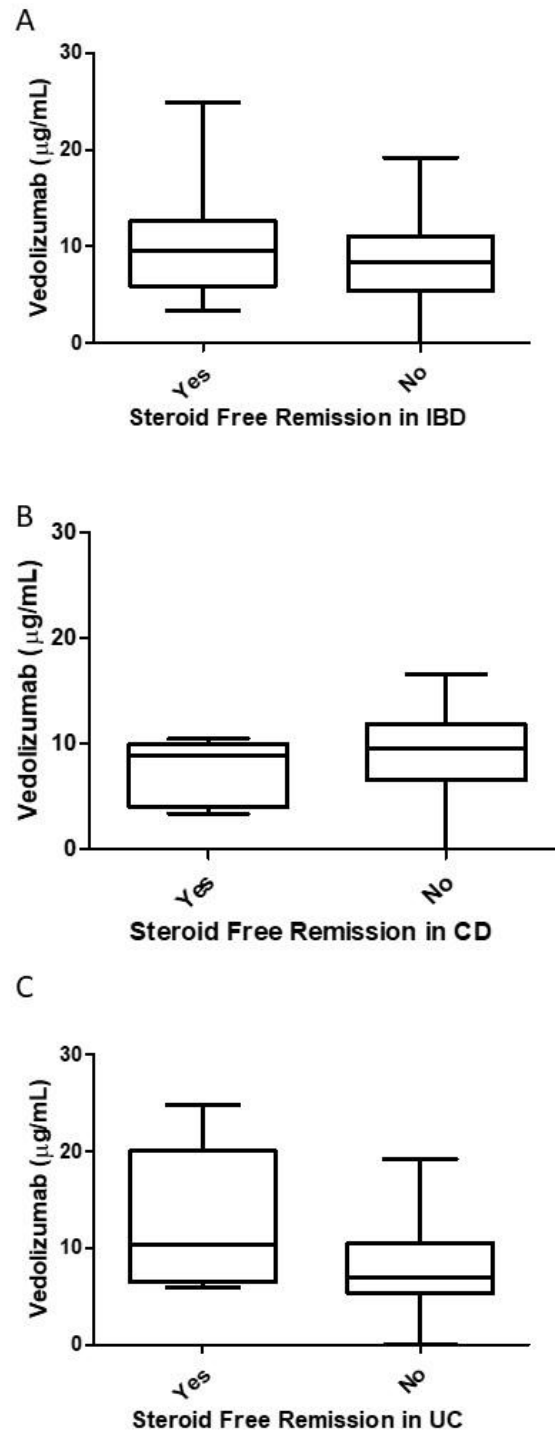


**Figure 2.2: Boxplots Showing the Median, IQR and Range of Vedolizumab Trough Levels ( $\mu\text{g/mL}$ ) of 34 Patients with IBD on Maintenance Therapy.**

The VDZ trough levels, median [range] of 34 patients with IBD (15 CD, 19 UC) during maintenance therapy, with and without SFR at time of sampling, are shown. **A)** IBD: trough levels of  $9.6\mu\text{g/mL}$  [ $3.4\text{--}24.9\mu\text{g/mL}$ ] and  $8.95\mu\text{g/mL}$  [ $0.0\text{--}19.2\mu\text{g/mL}$ ] respectively, **B)** Crohn's disease:  $8.4\mu\text{g/mL}$  [ $3.4\text{--}10.5\mu\text{g/mL}$ ] and  $9.6\mu\text{g/mL}$  [ $0.0\text{--}16.6\mu\text{g/mL}$ ] **C)** ulcerative colitis:  $11.2\mu\text{g/mL}$  [ $5.9\text{--}24.9\mu\text{g/mL}$ ] and  $6.9\mu\text{g/mL}$  [ $5.4\text{--}19.2\mu\text{g/mL}$ ]. Variables for those with and without SFR were compared with Mann Whitney U test. No significant difference between the groups was found.

#### *2.4.2.3 Maintenance study: Association of VDZ Trough Concentrations with Prior or Concomitant Medications*

Seven patients were receiving concomitant immunomodulators; three azathioprine (AZA) and four methotrexate (MTX). The median [range] VDZ trough levels for patients on and off concomitant AZA were 9.7µg/mL [5.9–7µg/mL] and 9.5µg/mL [0–24.9µg/mL] respectively. The median [range] VDZ trough levels for patients on and off concomitant MTX were 6.1µg/mL [4.3–9.5µg/mL] and 9.65µg/mL [0–24.9µg/mL] respectively. There was no significant difference in the trough concentrations obtained from patients related to concomitant therapy with either AZA or MTX.

The absence of detectable antibodies to VDZ in all 37 samples tested patients prevented assessment of any relationship between concomitant immunomodulator therapy and their presence.

Six patients were naïve to biologic agents prior to VDZ. Twenty-eight patients had received prior anti-TNF therapy. No patients had received other biologic agents (e.g., ustekinumab) or had exposure to Janus kinase-signal transducers and activators of transcription (JAK-STAT) inhibitors. Of the six biologic naïve patients, three were in remission at time of sampling (50%), compared with 11/28 patients who had prior anti-TNF therapy (42%). The median [range] VDZ trough levels for patients with and without prior anti-TNF therapy were not significantly different at 9.5µg/mL [0.0–21.1µg/mL] and 10.15µg/mL [5.8–24.9µg/mL] respectively (**Figure 2.3**).

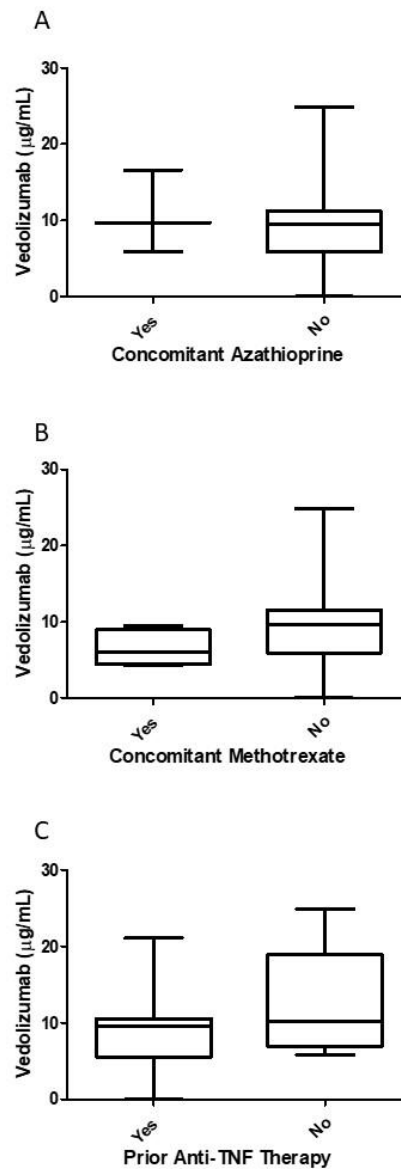


**Figure 2.3 Boxplots Showing the Median, IQR and Range of Vedolizumab Trough Levels (µg/mL) during maintenance therapy with and without other Prior or Concomitant IBD Therapies.**

The VDZ trough levels, median [range], for 34 patients on maintenance therapy by history of prior or concomitant drug use is shown. **A)** Patients with (n=3) and without (n=31) concomitant azathioprine; 9.7µg/mL [5.9–7µg/mL] and 9.5µg/mL [0–24.9µg/mL] respectively. **B)** Patients with (n=4) and without (n=30) concomitant methotrexate; 6.1 µg/mL [4.3–9.5µg/mL] and 9.65µg/mL [0–24.9µg/mL]. **C)** Patients with (n=28) and without (n=6) prior anti-TNF therapy; 9.5µg/mL [0.0–21.1µg/mL] and 10.15 µg/mL [5.8–24.9µg/mL]. Significance was assessed by Mann Whitney U test. No significant differences between the groups were found.

#### 2.4.2.4 Maintenance Study: Association of Trough VDZ Concentration with CRP, Albumin and Calprotectin

Pre-infusion CRP and albumin concentration levels were available for 21 patients. The median [range] pre-infusion CRP concentration was 3.4mg/L [0.3–28mg/L]. Eight patients had a CRP higher than 5mg/L. The median pre-infusion albumin concentration was 41g/L [39–48g/L]. No correlations between trough VDZ levels and either CRP or albumin levels were found with a correlation coefficient (r value) of -0.27 (p=0.25) and r=0.14 (p=0.54) respectively (**Figure 2.4**).

Thirteen patients had faecal samples obtained for calprotectin measurement within the 24 hours prior to their infusion; in six patients it was  $\geq 250\mu\text{g/g}$ . The median faecal calprotectin level was  $277\mu\text{g/g}$  [24–>1000 $\mu\text{g/g}$ ]. There was no significant difference in the trough VDZ levels of patients with calprotectin levels of  $\geq 250\mu\text{g/g}$  and those with levels  $< 250\mu\text{g/g}$  with VDZ median [range] trough concentrations  $7.85\mu\text{g/mL}$  [5.4–19.2 $\mu\text{g/mL}$ ] and  $6.5\mu\text{g/mL}$  [5.3–16.9 $\mu\text{g/mL}$ ] respectively. Only three patients had faecal calprotectin levels  $< 50\mu\text{g/g}$ . There was no significant difference between VDZ trough levels in patients with calprotectin levels  $\geq 50\mu\text{g/g}$  and those with levels  $< 50\mu\text{g/g}$  with VDZ median [range] trough concentrations of  $7.85\mu\text{g/mL}$  [5.4–19.2  $\mu\text{g/mL}$ ] and  $6.5\mu\text{g/mL}$  [5.3–16.9  $\mu\text{g/mL}$ ] respectively, p=0.387

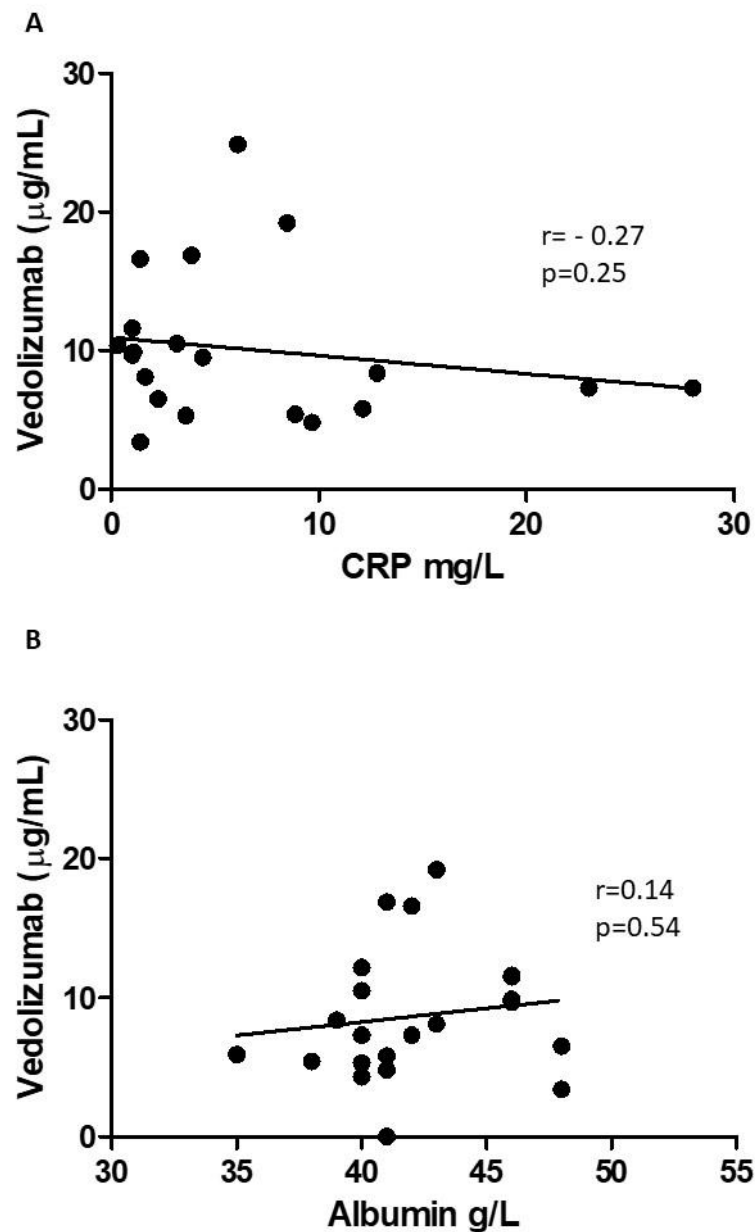


**Figure 2.4: Scatterplots Showing the Relationship between Vedolizumab Trough Concentration (µg/mL) during Maintenance Therapy and Concomitant Serum CRP (mg/L) and Albumin (g/L).** Pearson's Correlation coefficient was used to assess the relationship between VDZ and serum CRP and albumin. Samples for trough VDZ concentration, serum CRP, and albumin were obtained prior to a scheduled VDZ infusion during maintenance therapy. In **A**) the scatterplot shows the absence of correlation between the VDZ concentration (µg/mL) and CRP (mg/L),  $r = -0.27$ ,  $p = 0.25$  and in **B**) no correlation between VDZ concentration and serum albumin (g/L) is evident,  $r = 0.14$ ,  $p = 0.54$ .

### 2.4.3 Induction Study

#### 2.4.3.1 Induction Study Patient Characteristics

Of 44 patients recruited, four were excluded from analysis (one withdrew consent, one required colectomy within 48 hours of enrolment and two had no baseline samples). Of the remaining 40 included in the induction analysis, a further four defaulted prior the week 30 monitoring visit.

Patient demographics and characteristics are listed (**Table 2.2**). The median [range] age of 40 evaluable patients at time of first infusion was 51.95 years [18.2–75.8 years] with an almost equal number of male and female patients. The median baseline CRP was 3.6mg/L [1–43mg/L], albumin 41g/L [30–52g/L] and faecal calprotectin 764µg/g [16.4–1250µg/g]. Twenty-two of 40 (55%) patients had CD and 18 (45%) had UC.

Of 22 patients with CD, 12 had ileocolonic; six ileal; and four colonic involvement. Ten had non-stricturing, non-penetrating disease, eight had stricturing disease and four had penetrating disease. Perianal disease was present in nine and one patient had involvement of the upper gastrointestinal tract. The median [range] baseline Harvey Bradshaw Index (HBI) in CD patients was 7 [1–20].

Of 18 patients with UC, two thirds had extensive colitis and one third had left sided involvement. No patient in this cohort had proctitis. The median [range] baseline partial Mayo subscore was 4 [0–9].

The median [range] time from diagnosis to VDZ initiation in the 40 patients was 10.4 months [0.4–40.8]. One fifth (20%) of patients were receiving oral corticosteroids and 22% concomitant immunomodulators at time of VDZ initiation. This was therefore a treatment experienced group with only one third of the cohort naïve to biologic agents. Twenty-five percent had prior treatment with three or more biologic agents. No patient had prior exposure to JAK-STAT inhibitors.

| Cohort B: Vedolizumab Induction Study                                                                                                                                                                                         |                                                                                  |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------|
| <b>Patient Characteristics</b>                                                                                                                                                                                                | N=40                                                                             |
| <b>Age (years)</b><br>Median [range]                                                                                                                                                                                          | 51.95 [18.2–75.8]                                                                |
| <b>Gender</b><br>Female<br>Male                                                                                                                                                                                               | 19 (48%)<br>21 (52%)                                                             |
| <b>Disease Duration (years)</b>                                                                                                                                                                                               | 10.4 [0.4–40.8]                                                                  |
| <b>Crohn's Disease</b><br><i>Disease Extent (n=22)</i><br>Ileal<br>Colonic<br>Ileocolonic<br><i>Disease Behaviour (n=22)</i><br>Non-stricturing non-penetrating<br>Stricturing<br>Penetrating<br><i>Harvey Bradshaw Index</i> | 6 (27%)<br>4 (18%)<br>12 (54%)<br><br>10 (45%)<br>8 (36%)<br>4 (18%)<br>7 [1-20] |
| <b>Ulcerative colitis</b><br><i>Disease Extent (n=18)</i><br>Proctitis<br>Left-sided colitis<br>Extensive colitis<br><i>Partial Mayo Subscore</i>                                                                             | 0 (0%)<br>6 (33%)<br>12 (66%)<br>6 [0–9]                                         |
| <b>Laboratory parameters</b><br>Faecal calprotectin (µg/g) (n=29)<br>C-reactive protein (mg/L) (n=38)<br>Albumin (g/L) (n=39)                                                                                                 | 764 (6.4–1250)<br>3.6 [1–43]<br>41 [30–52]                                       |
| <b>5-Aminosalicylates</b>                                                                                                                                                                                                     | 18 (45%)                                                                         |
| <b>Oral Corticosteroids</b>                                                                                                                                                                                                   | 8 (20%)                                                                          |
| <b>Immunomodulators</b><br>Thiopurines<br>Methotrexate                                                                                                                                                                        | 9 (22.5%)<br>8 (20%)<br>1 (2.5%)                                                 |
| <b>Anti-TNF Therapy</b><br>Naïve<br>≥1 previous agent<br>≥2 previous agents<br>≥3 previous agents                                                                                                                             | 12 (30%)<br>28 (70%)<br>21 (52%)<br>10 (25%)                                     |

**Table 2.2. Demographics and Clinical Characteristics of Induction Study Participants (n=40) at Time of Vedolizumab Initiation**

\*Categorical variables presented as percentages

\*\*Continuous variables presented as mean [range]

#### 2.4.3.2. Induction Study: Treatment Duration

Between 15 May 2017 and 29 February 2019, 40 patients with IBD were included in the induction study. The date of last follow-up was 1 November 2020. The median duration of follow-up was 17.8 months [range 0.5–41 months] with complete treatment persistence data available for 38 patients to 20 months and 37 patients to 24 months. Two patients were lost to follow-up in the first year after the week 30 assessment and were excluded from the Kaplan-Meier survival analysis.

By week 14, 2/38 (5%) had failed VDZ therapy. As of week 30 (6.9 months), 8/38 (21%) patients had discontinued VDZ, two of whom required colectomy within the 30 weeks.

Of the 22 patients with CD, 9 (40%) were in SFR at week 14 and 6 (28%) at week 30. Of 18 patients with UC, 7 (38%) were in SFR at week 14 and 9 (50%) at week 30. There was no significant difference in the rates of SFR in CD and UC at week 14,  $p=0.6$  or week 30,  $p=0.2$ .

At 12 months, 24/38 (63%) IBD patients and at 24 months 17/37 (46%) were continuing VDZ. Twenty-three (61%) patients discontinued VDZ during the study; the median time to discontinuation was 9.33 months [range 0.5–38.7 months] (**Figure 2.5**). Reasons for discontinuation included primary nonresponse, 5 (22%), secondary loss of response, 10 (43%), intolerance or adverse event, 5 (22%), and reasons not stated, 3 (13%). Adverse events included three patients with joint pain, one with deteriorating renal function, and one with recurrent sinusitis

At enrolment 11/40 patients were naïve to biologic therapy and 29 had prior anti-TNF therapy. Of naïve patients 10/11 (91%) at 12 months and 8/11 (73%) at 24 months were continuing VDZ. The median time to discontinuation for three naïve patients was 15.8 months [range 5.4-16.4 months]. Of those with prior anti-TNF exposure 16/29 (55%) at 12 months and 11/29 (38%) at 24 months were continuing VDZ. The median time to VDZ discontinuation in anti-TNF treated patients was 8.2 months [range 0.5-38.7 months],  $p=0.054$ . (**Figure 2.6**).

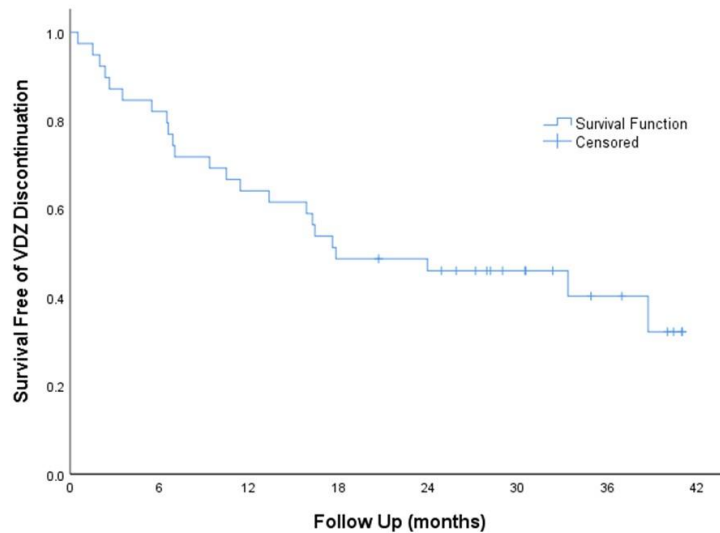


**Figure 2.5: Vedolizumab Persistence in the Induction Study Cohort.**

Kaplan-Meier curve demonstrating VDZ treatment continuation for 38 patients with 12 month data available. Complete 24 month data was available for 37 patients. At 12 months, 24/38 (63%) and at 24 months 17/37 (46%) continued treatment. Of 23/38 (61%) patients who discontinued VDZ during the study, the median [range] time to discontinuation was 9.33 months [0.5–38.7 months].

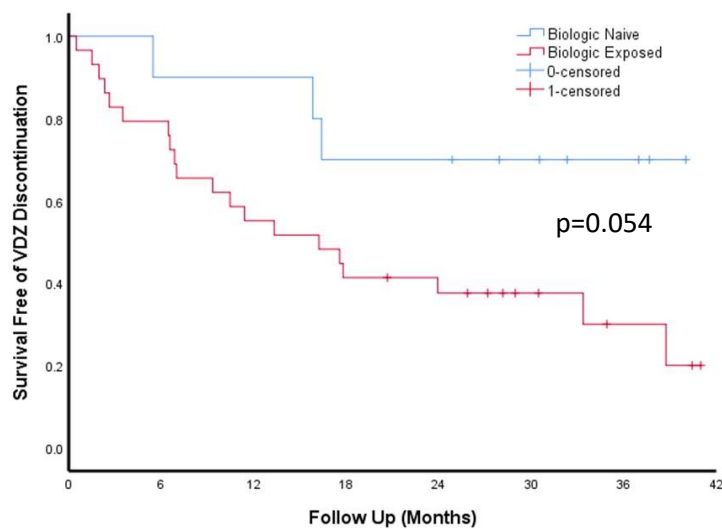


**Figure 2.6: Vedolizumab Persistence in anti-TNF Naïve and anti-TNF treated Patients.**

Kaplan-Meier curve demonstrating VDZ treatment continuation for 11 anti-TNF naïve and 29 anti-TNF treated patients. At 12 and 24 months respectively, 10/11 (91%) and 8/11 (73%) naïve and 16/29 (55%) and 11/29 (38%) anti-TNF treated patients were continuing VDZ. The median time to discontinuation for naïve patients was 15.8 months [range 5.4-16.4 months] and for anti-TNF treated patients 8.2 months [range 0.5-38.7 months] The difference was evaluated using the log-rank test.

#### 2.4.3.3 Induction Study: Vedolizumab

##### 2.4.3.3.1 Vedolizumab: Peak and Trough concentrations

Vedolizumab concentrations are presented as median [range]. VDZ peak concentration levels were available for 37, 36, 37 and 36 of patients for the first, second, third and fourth infusions respectively. The VDZ peak concentrations 30 minutes post-infusion were 94.6µg/mL [42.5-166µg/mL], 125.6µg/mL [48.3–200µg/mL], 120.9µg/mL [58.8–200.0µg/mL], and 104.3µg/mL [55.1–200µg/mL] for their first, second, third, and fourth infusions respectively. Of 36 patients who completed induction and tested for antibodies to VDZ immediately before their fourth infusion, none were detected.

Vedolizumab trough concentration levels were available for 36, 37 and 36 of patients prior to the second, third and fourth infusion respectively. The pre-infusion VDZ trough concentrations for second, third and fourth infusions were 25.1µg/mL [0–56µg/mL], 20.2µg/mL [0–66µg/mL], and 12.5µg/mL [0–38.8µg/mL] respectively (**Figure 2.6**). The decreasing levels can be attributed to the lengthening interval between doses, two weeks between the first and second infusions, four weeks between the second and third and four or eight weeks between the third and fourth infusions for patients with CD and UC respectively.

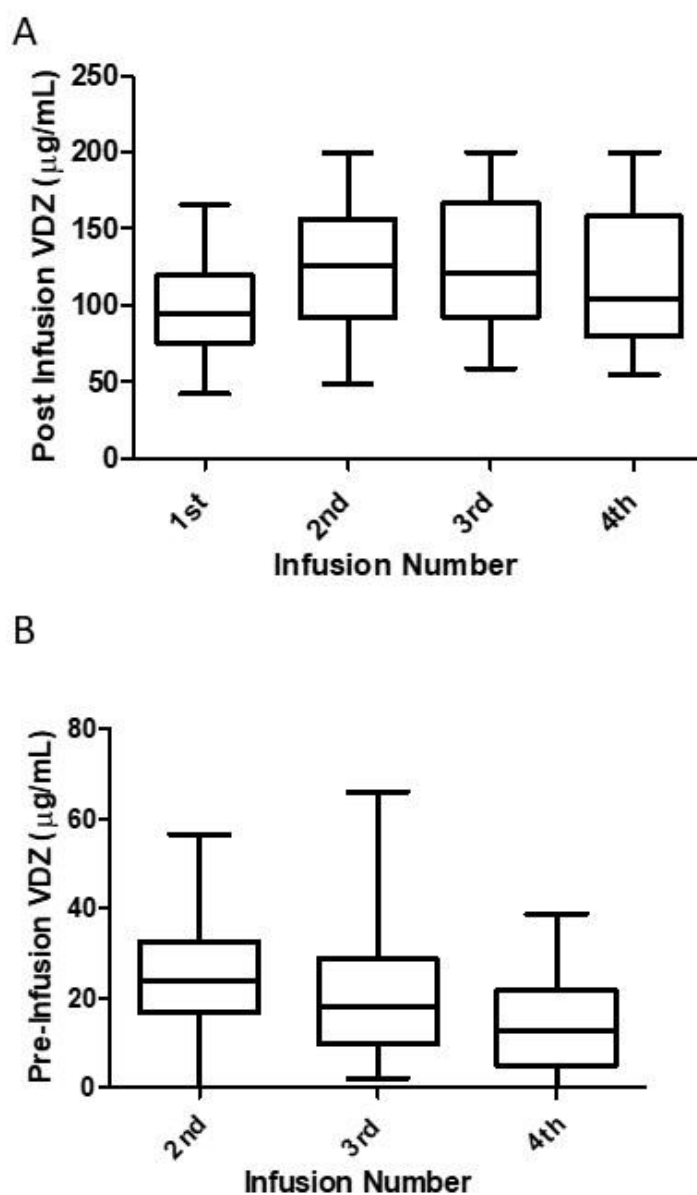


**Figure 2.7 Boxplots Showing the Median, IQR and Range of Vedolizumab Serum Concentrations ( $\mu\text{g/mL}$ ) During Induction Therapy**

Post- and Pre-infusion VDZ concentration levels in 40 patients with IBD (22 CD, 18 UC). The 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> infusions were at weeks 0, 2, 6 and either week 10 (CD) or week 14 (UC). **A)** The median [range] concentrations in serum obtained 30 minutes following completion of each infusion were 94.6 $\mu\text{g/mL}$  [42.5–166 $\mu\text{g/mL}$ ], 125.6 $\mu\text{g/mL}$  [48.3–200 $\mu\text{g/mL}$ ], 120.9 $\mu\text{g/g}$  [58.8–200.0 $\mu\text{g/mL}$ ], and 104.3 $\mu\text{g/mL}$  [55.1–200 $\mu\text{g/mL}$ ] for their first, second, third, and fourth infusions respectively. **B)** The median [range] trough concentrations, obtained immediately prior to the second, third and fourth infusions, were 25.1 $\mu\text{g/mL}$  [0–56 $\mu\text{g/mL}$ ], 20.2 $\mu\text{g/mL}$  [0–66 $\mu\text{g/mL}$ ], and 12.5 $\mu\text{g/mL}$  [0–38.8 $\mu\text{g/mL}$ ] respectively.

#### 2.4.3.3.1.1 Vedolizumab: Peak and Trough concentrations by Disease Phenotype

The median [range] of week 2 VDZ trough concentrations for 22 patients with CD was 22.8µg/mL [0–52.7µg/mL] and did not differ from that in 18 patients with UC, 25.4µg/mL [14.6–56.6µg/mL]  $p=0.339$ . Week 6 VDZ trough concentrations were lower in 19 patients with CD 12.4µg/mL [2.0–39.2µg/mL] compared with 18 patients with UC, 21.95µg/mL [5.1–66µg/mL]  $p=0.047$ . **Figure 2.8.**

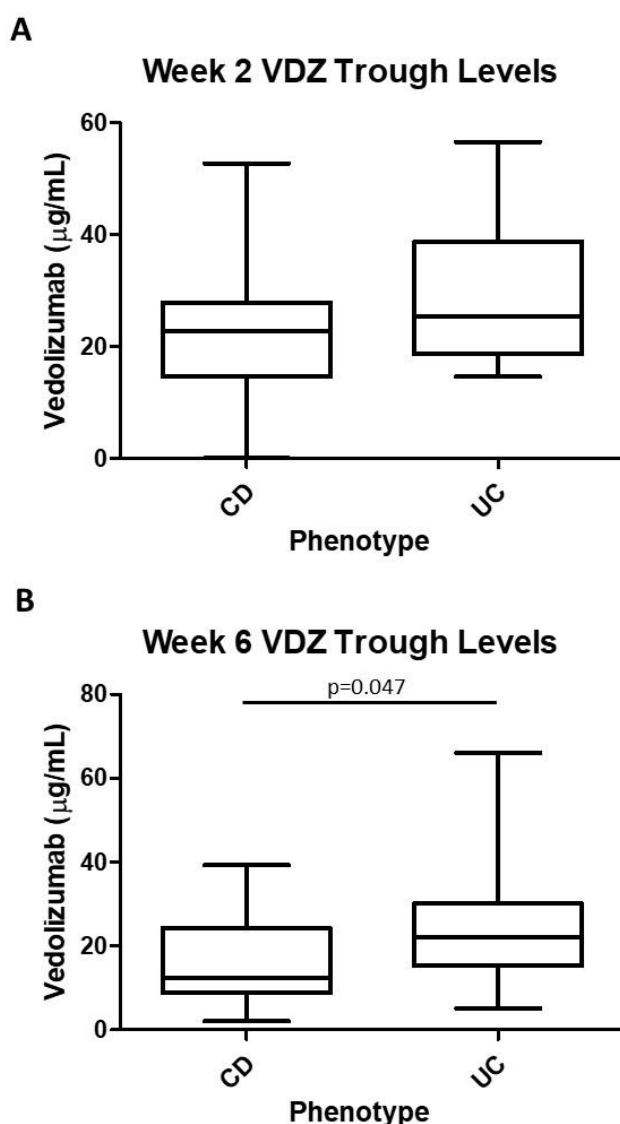


**Figure 2.8 Boxplots Showing the Median, IQR, and Range of Vedolizumab Trough Concentrations (µg/mL) at Weeks 2 and 6 for patients with CD and UC**

VDZ trough levels in for patients with CD and UC. **A)** at week 2, 22.8µg/mL [0–56.6µg/mL] and 25.6µg/mL [14.1–52.7µg/mL] respectively,  $p=NS$ . **B)** at week 30; 25.4µg/mL [15.5–56.6µg/mL] and 21.0µg/mL [0–52.7µg/mL] respectively,  $p=0.047$ . Significance was assessed by Mann Whitney U test

#### 2.4.3.3.2 Vedolizumab Trough Concentrations and Steroid-Free Remission (SFR) at Weeks 14 and 30

Following induction, 18/40 (45%) patients at week 14 and 15/40 patients (40%) at week 30 were in SFR. There were no significant differences in SFR rates by disease phenotype. At week 14 10/22 (45%) patients with CD and 8/18 (44%) with UC were in SFR,  $p=0.601$ . At week 30, 7/22 (32%) patients with CD and 9/18 (50%) with UC were in SFR,  $p=0.2$ .

Analysis of the total IBD cohort found no difference between week 2 trough VDZ levels in those without and those with SFR at week 14; 25.6 $\mu$ g/mL [14.1-52.7 $\mu$ g/mL] and 22.8 $\mu$ g/mL [0-56.6 $\mu$ g/mL], or at week 30; 21.0 $\mu$ g/mL [0-52.7 $\mu$ g/mL] and 25.4 $\mu$ g/mL [15.5-56.6 $\mu$ g/mL],  $p=NS$ , (**Figure 2.7**, panels A and B). Week 6 trough levels were significantly higher in those with SFR compared to those without SFR at week 14; 24 $\mu$ g/mL [2.4-55.8mL] and 14.0 $\mu$ g/mL [2.0-66.0/mL],  $p=0.015$ , and at week 30; 28.3/mL [8.8-55.0 $\mu$ g/mL] and 12.9 $\mu$ g/mL [2.0-66 $\mu$ g/mL],  $p=0.029$ , respectively (**Figure 2.7**, panels C and D).

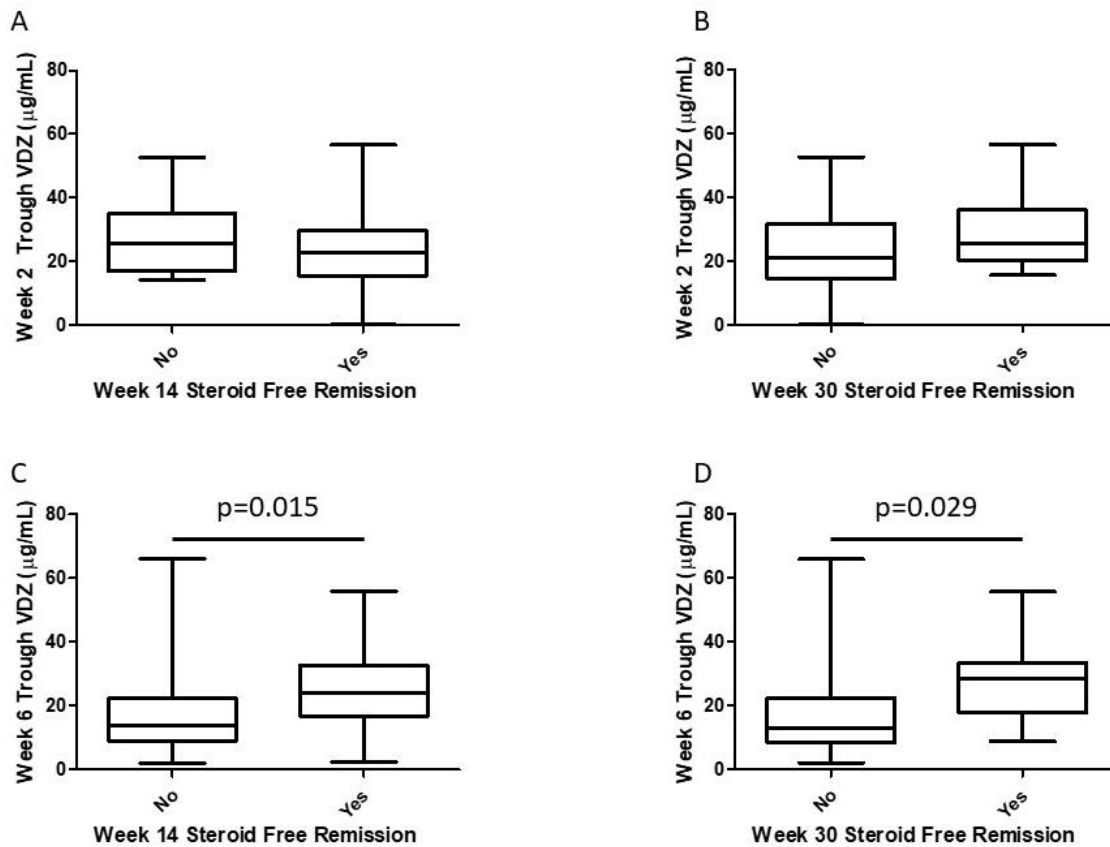


**Figure 2.9 Boxplots Showing the Median, IQR, and Range of Vedolizumab Trough Concentrations ( $\mu\text{g/mL}$ ) at Weeks 2 and 6 in Those With and Without Steroid Free Remission at Weeks 14 and 30.**

Week 2 VDZ trough levels, median [range] are shown for those without and with SFR in **A**) at week 14; 25.6 $\mu\text{g/mL}$  [14.1-52.7 $\mu\text{g/mL}$ ] and 22.8 $\mu\text{g/mL}$  [0-56.6 $\mu\text{g/mL}$ ],  $p=\text{NS}$ , and in **B**) at week 30; 21.0 $\mu\text{g/mL}$  [0-52.7 $\mu\text{g/mL}$ ] and 25.4 $\mu\text{g/mL}$  [15.5-56.6 $\mu\text{g/mL}$ ],  $p=\text{NS}$  respectively.

Week 6 VDZ trough levels, median [range] are shown for those without and with SFR in **C**) at week 14; 14.0 $\mu\text{g/mL}$  [2.0-66.0 $\mu\text{g/mL}$ ] and 24 $\mu\text{g/mL}$  [2.4-55.8 $\mu\text{g/mL}$ ],  $p=0.015$ , and in **D**) at week 30; 12.9 $\mu\text{g/mL}$  [2.0-66 $\mu\text{g/mL}$ ] and 28.3 $\mu\text{g/mL}$  [8.8-55.0 $\mu\text{g/mL}$ ],  $p=0.029$ , respectively.

Significance was assessed by Mann Whitney U test

#### 2.4.3.3.2.1 Vedolizumab Trough Concentrations and Steroid-Free Remission (SFR) by Disease Phenotype

In a post hoc subgroup exploratory analysis patients were stratified based on IBD phenotype. Week 2 and week 6 pre infusion VDZ trough levels for CD and UC were compared. Of 22 patients with CD, 19 had week 2 VDZ trough levels available. Eight (42%) were in SFR at week 14 and six (32%) at week 30. Nineteen patients had week 6 VDZ levels; nine (48%) were in SFR at week 14 and six (32%) at week 30. Week 2 and week 6 pre infusion trough levels were not associated with either week 14 SFR,  $p=0.77$  and  $p=0.141$  respectively, or with week 30 SFR,  $p=0.058$  and  $p=0.087$  respectively.

Fifteen of 18 patients with UC had week 2 VDZ trough levels available. Seven were in SFR at week 14 (47%) and nine (60%) at week 30. All 18 patients with UC had VDZ trough levels at week 6; eight (44%) were in SFR at week 14 and 9 (50%) were in SFR at week 30. Week 2 and week 6 pre infusion trough levels were not associated with either week 14 SFR,  $p=0.635$  and  $p=0.11$  respectively, or with week 30 SFR,  $p=0.95$  and  $p=0.063$  respectively. (Table 2.3)

| <b>Trough VDZ Concentrations (median [range]) and Disease Activity at weeks 14 and 30 by Disease Phenotype</b> |                               |             |                |                               |             |                |
|----------------------------------------------------------------------------------------------------------------|-------------------------------|-------------|----------------|-------------------------------|-------------|----------------|
| <b><i>Crohn's Disease (N22)</i></b>                                                                            | <b>Disease status Week 14</b> |             | <b>p value</b> | <b>Disease status Week 30</b> |             | <b>p value</b> |
| Week 2 (N19)*                                                                                                  | Flare (N11)                   | SFR (N8)    |                | Flare (N13)                   | SFR (N6)    |                |
| VDZ level (pg/mL)                                                                                              | 21                            | 23.8        | 0.77           | 19.2                          | 26.75       | 0.058          |
|                                                                                                                | [14.1-52.7]                   | [0-47]      |                | [0-52.7]                      | 22.8-47]    |                |
| Week 6 (N19)*                                                                                                  | Flare (N10)                   | SFR (N9)    |                | Flare (N 13)                  | SFR (N 6)   |                |
| VDZ level (pg/mL)                                                                                              | 10.7                          | 22.8        | 0.141          | 9.9                           | 23.1        | 0.087          |
|                                                                                                                | [2-24.2]                      | [2.4-39.2]  |                | [2-36.3]                      | [8.8-39.2]  |                |
| <b><i>Ulcerative colitis (N18)</i></b>                                                                         | <b>Disease status Week 14</b> |             |                | <b>Disease status Week 30</b> |             |                |
| Week 2 (N15)**                                                                                                 | Flare (N8)                    | SFR (N7)    |                | Flare (N6)                    | SFR (N9)    |                |
| VDZ level (pg/mL)                                                                                              | 30.95                         | 20.9        | 0.635          | 30.0                          | 20.9        | 0.95           |
|                                                                                                                | [14.6-45.6]                   | [15.5-56.6] |                | 14.6-45.6-                    | 15.5.55.8   |                |
| Week 6 (N18)**                                                                                                 | Flare (N10)                   | SFR (N 8)   |                | Flare (N 9)                   | SFR (N 9)   |                |
| VDZ level (pg/mL)                                                                                              | 13.4                          | 14.5        | 0.11           | 15.5                          | 29.1        | 0.063          |
|                                                                                                                | [2.9-21.7]                    | [0-35.9]    |                | [5.1-66]                      | [15.6-55.8] |                |

**Table 2.3: Vedolizumab trough concentrations (week 2 and week 6) in patients with and without steroid free remission at weeks 14 and 30 stratified by disease phenotype**

\*Of 22 patients with CD, 19 had VDZ trough levels at week 2 and 19 at week 6

\*\* Of 18 patients with UC, 15 had VDZ trough levels at week 2 and 18 at week 6

#### 2.4.3.3.3 Receiver Operating Characteristics Curve Analyses to Define Vedolizumab Trough Concentration Predictive of Steroid-Free Remission

Receiver operating characteristics (ROC) curves plotted for Week 2 VDZ trough levels did not show a relationship with week 14 SFR. However, ROC curves plotted for Week 6 VDZ trough levels showed a relationship with week 14 SFR with the Area Under the Curve (AUC) of 0.704 [95% CI 0.53–0.879],  $p=0.034$ . A cut-off VDZ level of  $\geq 15.5$   $\mu\text{g/mL}$  gave a sensitivity of 82.5% and specificity of 65% for week 14 SFR (**Figure 2.8**). When the same threshold of  $\geq 15.5\mu\text{g/mL}$  was used to predict week 30 SFR the AUC was 0.771 [95%CI 0.616–0.993], sensitivity 86.7% and specificity 63.6%,  $p=0.006$ .

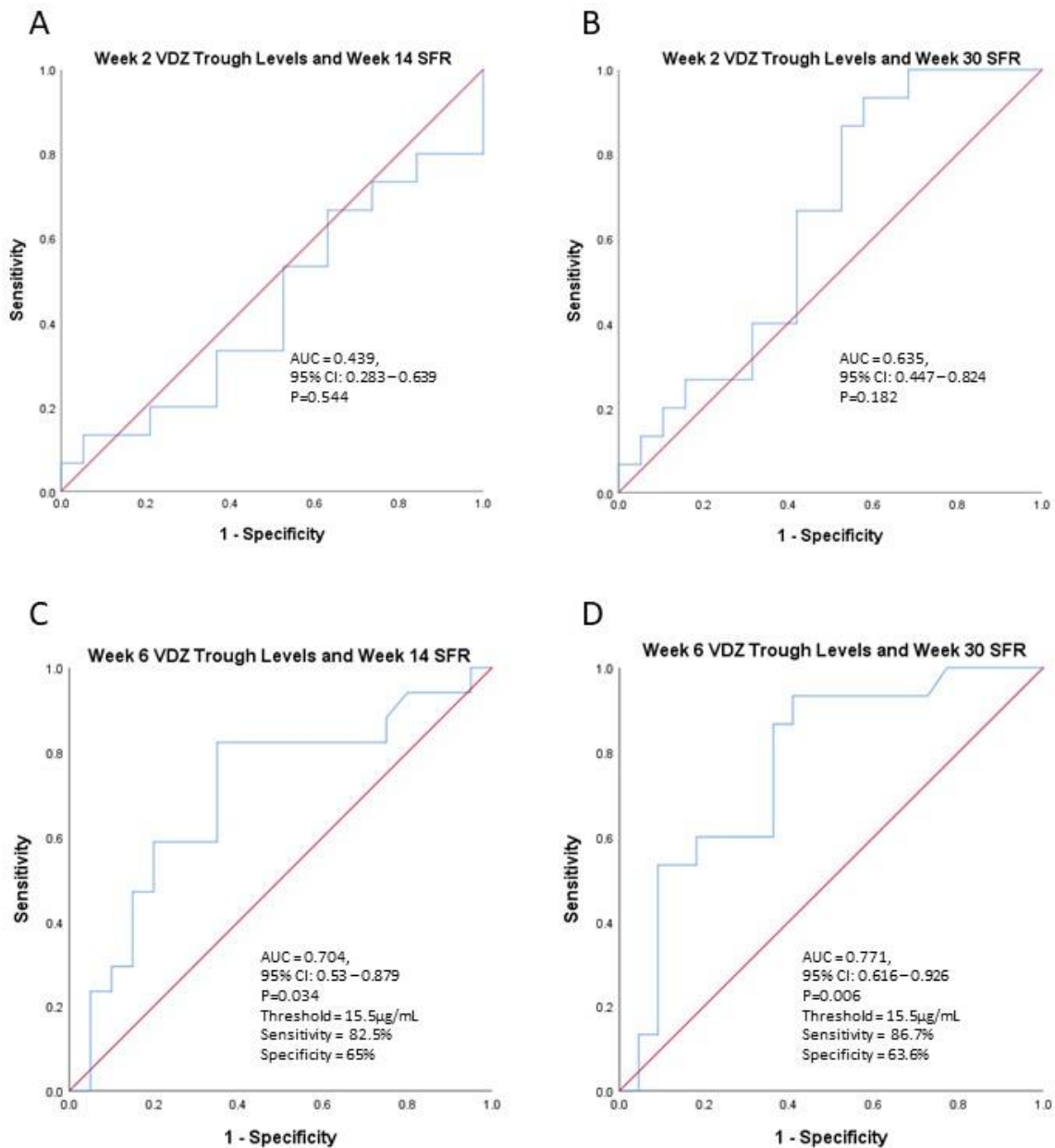


**Figure 2.10: Receiver Operating Characteristic (ROC) Curve of Vedolizumab Trough Levels as Predictors Steroid Free Remission**

Receiver-operating characteristic (ROC) curve statistics were used to determine the optimum VDZ trough level cut-off to predict steroid free remission (SFR). ROC curves of Week 2 VDZ trough levels do not show any relationship with week 14 **A)** or week 30 **B)** SFR; AUC 0.439 [95% CI 0.283-0.639],  $p=0.544$  and AUC 0.635 [95%CI 0.447-0.824],  $p=0.182$  respectively.

**C)** ROC analysis of week 6 trough levels for week 14 SFR, showed an AUC 0.704, [95%CI 0.53-0.879]. A VDZ threshold of  $\geq 15.5$  µg/mL was identified as predictive of week 14 SFR, sensitivity 82.5%, specificity 65%,  $p=0.017$ . **D)** ROC analysis of week 6 trough levels for week 30 SFR showed a AUC 0.771, [95% CI 0.616-0.92]. A week 6 VDZ trough level of  $\geq 15.5$  µg/mL was also predictive of week 30 SFR; , sensitivity 86.7%, specificity 63.6%,  $p=0.006$ .

#### 2.4.3.3.4 Vedolizumab Trough Concentration and Persistence on Therapy

A VDZ trough concentration of  $12\mu\text{g/mL}$  or greater is considered adequate during maintenance therapy.(341) In this induction study, a week 6 VDZ trough concentration of  $\geq 15.5\mu\text{g/mL}$  was associated with week 14 and with week 30 SFR. As persistence on therapy is an alternate indicator of disease outcome that takes into account real world pressures that may not always be fully captured by biochemical data or disease activity scores, we examined whether a week 6 VDZ trough of  $\geq 15.5\mu\text{g/mL}$  also associates with persistence on therapy

In 37/40 patients with data prospectively gathered from time of VDZ initiation that included a week 6 VDZ trough level, a trough level of  $\geq 15.5\mu\text{g/mL}$  (present in 22/37) was associated with significantly higher likelihood of remaining on VDZ therapy for at least 41 months ( $p=0.004$ ) (**Figure 2.9**).

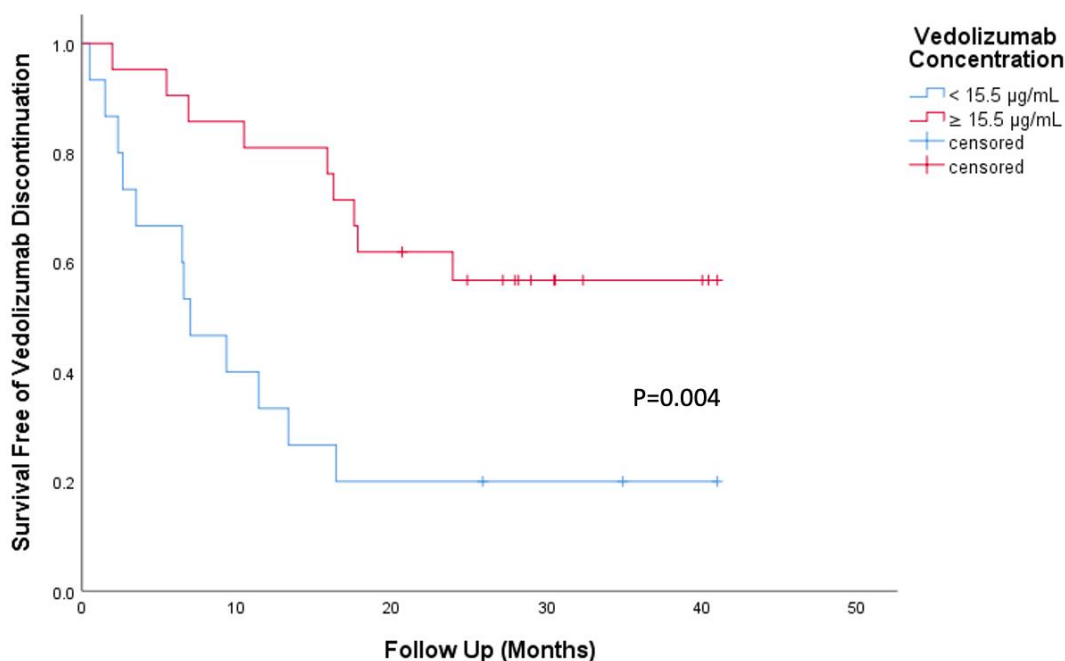


**Figure: 2.11: Kaplan-Meier Survival Curves Showing Survival Free of Vedolizumab Discontinuation in Those with Week 6 Vedolizumab Trough Concentrations  $\geq 15.5\mu\text{g/mL}$  (shown in red) Compared with Those with a Trough Concentration  $< 15.5\mu\text{g/mL}$  (shown in blue).**

Kaplan-Meier curves demonstrating persistence on VDZ for 37 patients, 22 with a week 6 trough level  $\geq 15.5\mu\text{g/mL}$  (shown in red) and 15 with a level  $< 15\mu\text{g/mL}$  (shown in blue). The difference was evaluated using the log-rank test.

#### 2.4.3.4 Vedolizumab Induction and Biomarkers of Disease Activity: C-Reactive Protein, Serum Albumin, and Faecal Calprotectin

##### 2.4.3.4.1 Vedolizumab Induction and CRP

CRP was not affected by VDZ during induction therapy. The median [range] CRP concentrations at baseline, and at the 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> infusion time-points were 3.6mg/L [1–43mg/L], 4.8mg/L [1–26mg/L], 3.5mg/L [1–89mg/L], and 4.0mg/L [1–72mg/L] respectively (**Figure 2.10**).

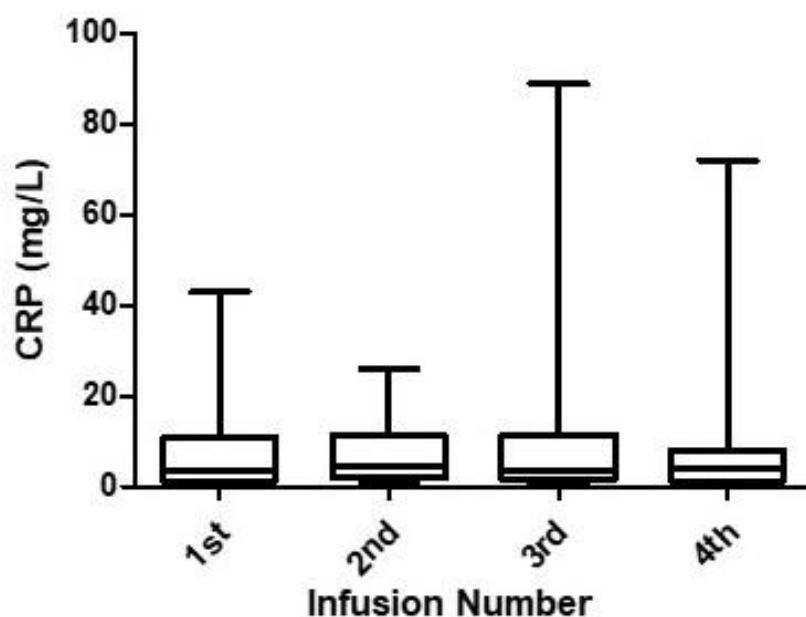


**Figure 2.12: Serum CRP during Vedolizumab Induction at Time of each Infusion**

Serum CRP levels in 40 patients with IBD (22 CD, 18 UC) were obtained prior to each infusion. The 1<sup>st</sup>, 2<sup>nd</sup>, 3<sup>rd</sup>, and 4<sup>th</sup> infusions were at weeks 0, 2, 6 and either week 10 (CD) or week 14 (UC). No consistent effect of VDZ on CRP was detected. There was no significant change in CRP between first and second, or first and third infusion. Significance was assessed using the Wilcoxon Sign Ranked Test.

#### 2.4.3.4.2 CRP and Vedolizumab Trough Concentrations

Fifteen patients had a baseline CRP of  $\geq 5\text{mg/L}$ . In 23 patients, the baseline CRP was  $<5\text{mg/L}$ . The median [range] week 6 VDZ trough concentration was significantly higher at  $22.5\mu\text{g/mL}$  [ $15.5\text{-}28.9\mu\text{g/mL}$ ] in those with CRP  $<5\text{mg/L}$  compared to those with a CRP  $\geq 5\text{mg/L}$ ,  $9.9\mu\text{g/mL}$  [ $6.5\text{-}20.4\mu\text{g/mL}$ ],  $p=0.01$  (Figure 2.11).

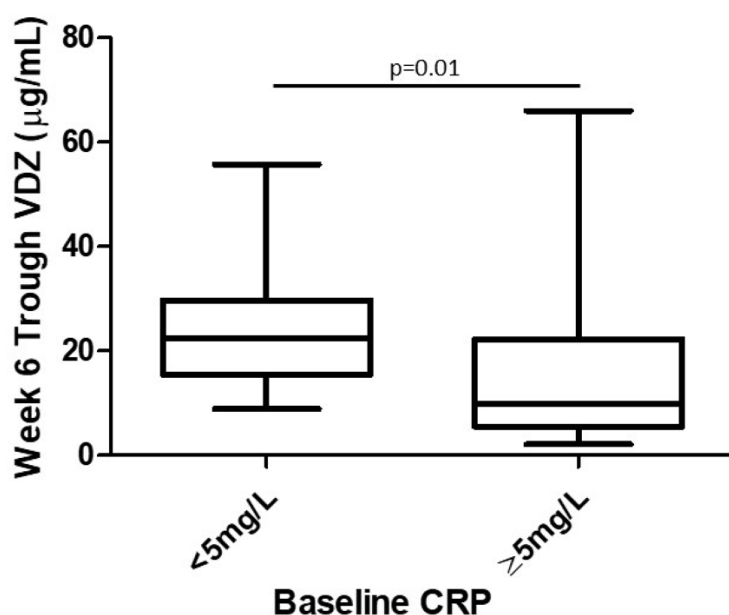


**Figure 2.13: Boxplot Showing the Median, IQR and Range of Week 6 Vedolizumab Trough Levels ( $\mu\text{g/mL}$ ) in Patients with a Baseline CRP $<5\text{mg/L}$  and  $\geq 5\text{mg/L}$ .**

The median [range] VDZ trough levels obtained immediately prior to the week 6 VDZ infusion were significantly higher in 23 patients with a baseline CRP $<5\text{mg/L}$  compared to 15 patients with a baseline CRP $\geq 5\text{mg/L}$ ;  $22.5\mu\text{g/mL}$  [ $15.5\text{-}28.9\mu\text{g/mL}$ ] compared to  $9.9\mu\text{g/mL}$  [ $6.5\text{-}20.4\mu\text{g/mL}$ ] respectively  $p=0.01$ . The Mann Whitney U test was used for the comparison.

On examining the relationships between the concomitant VDZ trough concentration and CRP levels, no correlation between week 2 VDZ trough levels and CRP was found. However, a moderately strong correlation between the trough VDZ level and the concomitant CRP was found at the third (week 6) and the fourth infusions (week 10 CD, week 14 UC) ( $r=-0.492$ ,  $p=0.005$ , and  $r=0.489$ ,  $p=0.006$  respectively) (Figure 2.12).

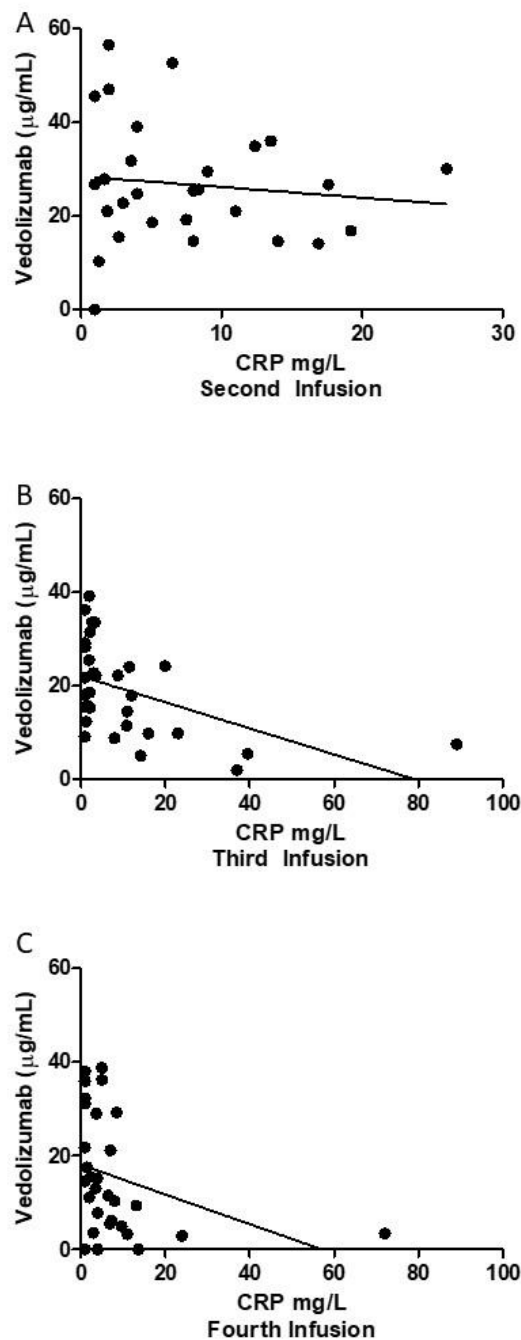


**Figure 2.14: Scatterplots Showing the Relationship between Vedolizumab Trough Levels (µg/mL) and the Concomitant CRP (mg/L) at the Second, Third and Fourth Vedolizumab Infusions.**

Pearson's Correlation coefficient was used to assess the relationship between VDZ trough concentrations and concomitant CRP levels. The scatterplots show In **A)** the lack of correlation at week 2,  $r=-0.1$ ,  $p=NS$ , in **B)** a moderately strong, negative, linear association between week 6 VDZ concentration and CRP level is shown,  $r= -0.492$ ,  $p=0.005$ , and in **C)** a moderately strong negative linear correlation between VDZ concentration for samples obtained immediately before the fourth infusion (weeks 10 or 14) is shown,  $r= -0.489$ ,  $p=0.006$ .

#### 2.4.3.4.3 Baseline CRP and Week 14 and Week 30 Steroid-Free Remission

Of 40 patients, 18 (45%) were in SFR at week 14 and 15 (40%) at week 30. Elevation of baseline CRP was significantly associated with absence of SFR at week 14, although not week 30 despite an evident trend in that direction. Of those in SFR at week 14, their baseline CRP (median [range]) was 2.0mg/L [1-12.5mg/L] compared with 8.8mg/L [1-43mg/L] for those with persistent disease activity,  $p=0.001$ . Of those in SFR at week 30, the baseline CRP was 3.0mg/L [1-16mg/L] compared with 6.9mg/L [1-43mg/L] in those with persistent disease,  $p=0.055$  (**Figure 2.13**).

Patients with a baseline CRP of  $\geq 5$ mg/L were less likely to be in steroid-free remission at week 14 (Odds Ratio (OR) 0.082, 95% CI 0.015–0.457,  $p=0.002$ ) or at week 30 SFR (OR 0.192, 95%CI 0.04–0.871  $p=0.026$ ).

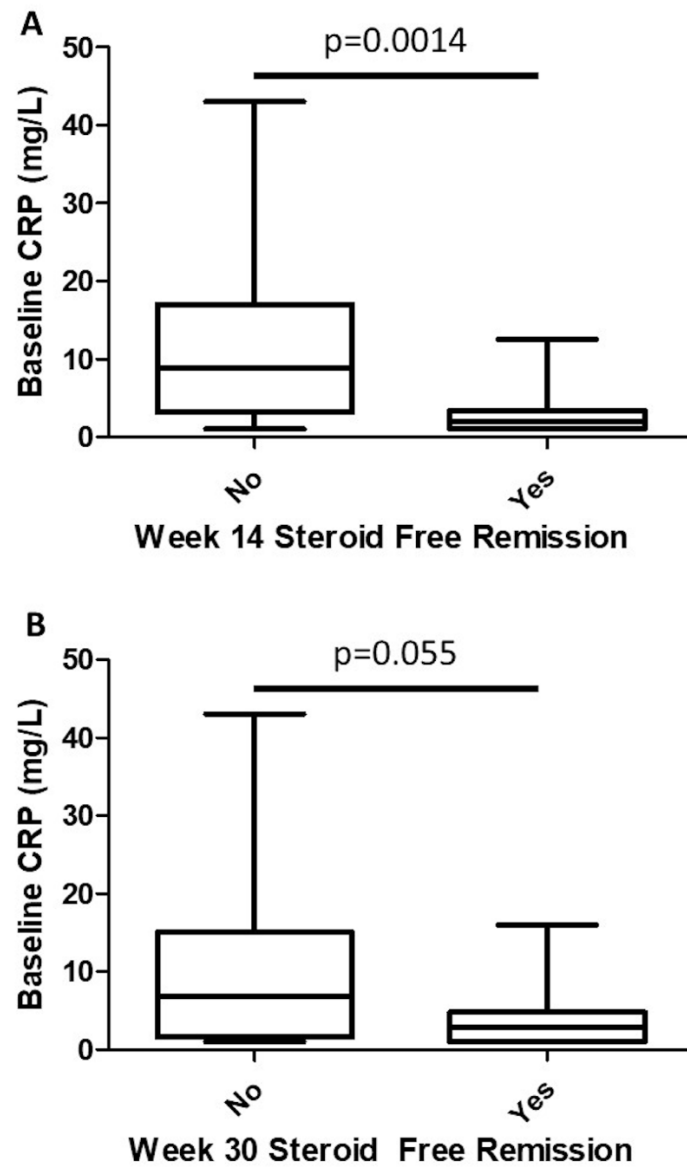


**Figure 2.15: Boxplots Showing the Median, IQR and Range of Baseline CRP (mg/L) in Those With and Without Steroid Free Remission at Week 14 and Week 30.**

In **A**) the baseline CRP, median [range], of 18 patients in SFR at week 14, 2.0mg/L [1-12.5mg/L] is compared to that of 22 patients with persistent disease, 8.8mg/L [1-43mg/L],  $p=0.001$ . In **B**) the baseline CRP of 15 patients in SFR at week 30, 3.0mg/L [1-16mg/L] is compared to that of those with persistent disease 6.9mg/L [1-43mg/L],  $p=0.055$ .

#### 2.4.3.4.4 Vedolizumab Induction and Serum Albumin

The association of baseline serum albumin with the week 6 VDZ trough level was examined. A serum albumin of  $\geq 40\text{g/L}$  at baseline was associated with higher week 6 drug levels (**Figure 2.14**). In 27/38 patients with baseline serum albumin  $\geq 40\text{g/L}$  the week 6 median [range] VDZ trough level was  $23.1\text{g/L}$  [ $14.8\text{-}31.0\text{g/L}$ ] compared with  $9.8\text{g/L}$  [ $8.2\text{-}16.6\text{g/L}$ ] in those with a baseline serum albumin level  $<40\text{g/dL}$ ,  $p=0.001$ .

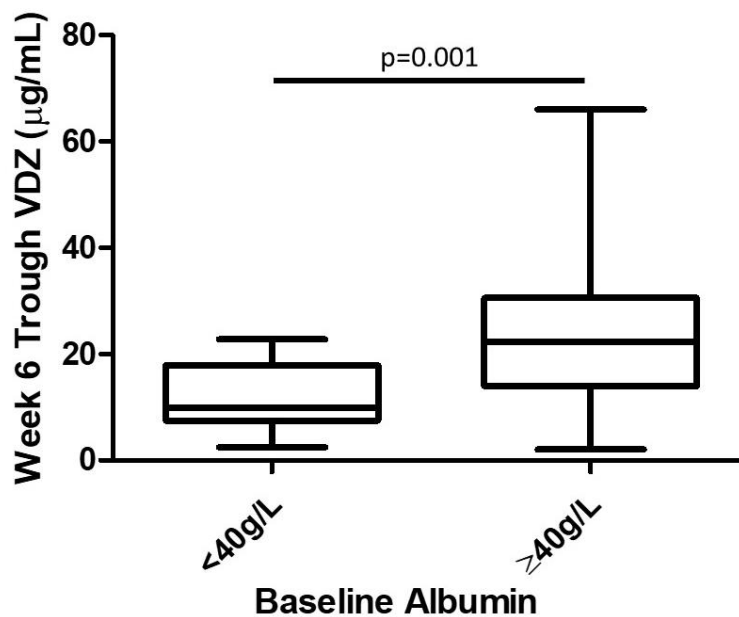


**Figure 2.16: Boxplots Showing the Median, IQR and Range of Week 6 Vedolizumab Trough Concentrations ( $\mu\text{g/mL}$ ) in Those With Baseline Serum Albumin  $<40\text{g/L}$  and  $\geq 40\text{g/L}$ .**

Vedolizumab trough levels obtained immediately prior to the week 6 VDZ infusion in 11 patients with baseline serum albumin  $<40\text{g/L}$ , ( $9.8\mu\text{g/mL}$  [range  $8.2\text{-}16.6\mu\text{g/mL}$ ]) and in 27 patients with a baseline serum albumin of  $\geq 40\text{g/L}$ , ( $23.1\mu\text{g/mL}$  [ $14.8\text{-}31.0\mu\text{g/mL}$ ]), are shown  $p=0.001$ . The Mann Whitney U test was used for the comparison.

A positive linear correlation between the concomitant pre-infusion serum albumin levels and VDZ trough concentrations at all time-points assessed was found (**Figure 2.15**). The correlation at week 2 (second infusion) and week 6 (third infusion ) was moderately strong,  $r=0.466$   $p=0.012$  and  $r=0.412$   $p=0.019$ , respectively. By the fourth infusion (week 10 (CD) or week 14 (UC) a weaker correlation was found,  $r=0.365$   $p=0.044$ .

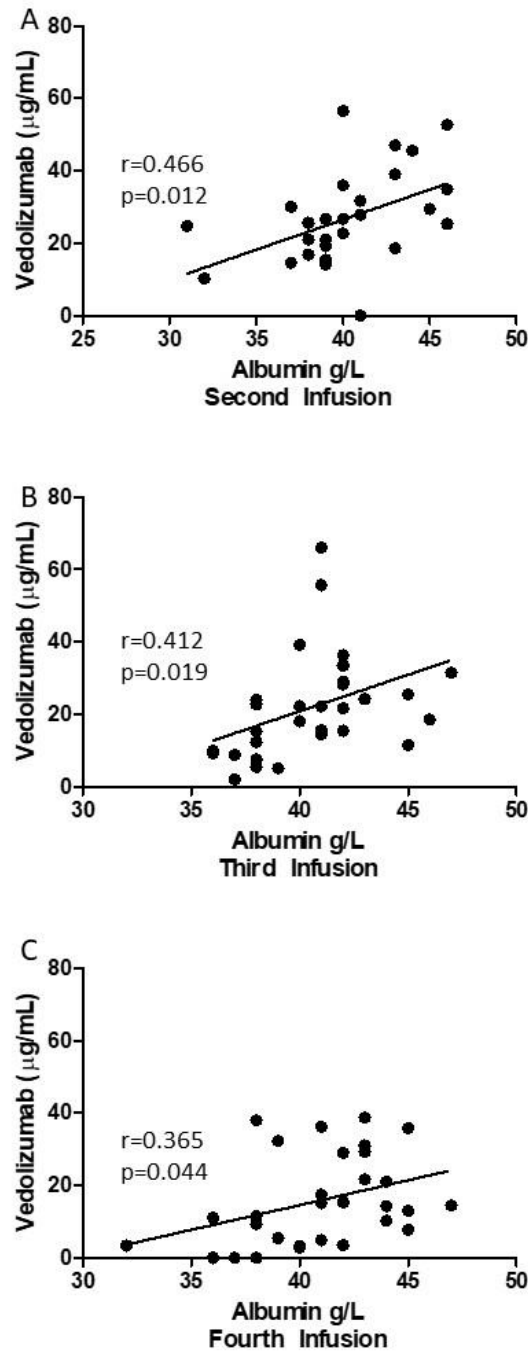


**Figure 2.17: Scatterplots Showing the Relationship between Vedolizumab Trough Concentration ( $\mu\text{g/mL}$ ) and the Concomitant Serum Albumin ( $\text{g/L}$ ) Prior to the Second, Third and Fourth Vedolizumab Infusions.**

Pearson's Correlation coefficient was used to assess the relationship between VDZ trough level and serum albumin. Samples for trough VDZ concentration and albumin were obtained prior to the second, third and fourth infusions. A positive linear correlation, between VDZ trough level and serum albumin at **A**) the second (week 2,  $r=0.466$ ,  $p=0.012$ ), **B**) the third (week 6,  $r=0.412$ ,  $p=0.019$ ) and **C**) the fourth (week 10 or 14,  $r=0.365$ ,  $p=0.044$ ) infusions. The correlation was of moderate strength at the second and third infusions and weak at the fourth infusion.

Baseline serum albumin did not associate with either week 14 or week 30 SFR. Of those in SFR at week 14, their baseline albumin (median [range]) was 42g/L [30-52g/L] compared with 41g/L [31-47mg/L] for those with persistent disease activity,  $p>0.05$ . Of those in SFR at week 30, the baseline albumin was 42g/L [34-52g/L] compared with 40g/L [30-47g/L] in those with persistent disease,  $p>0.05$  (Figure 2.16).

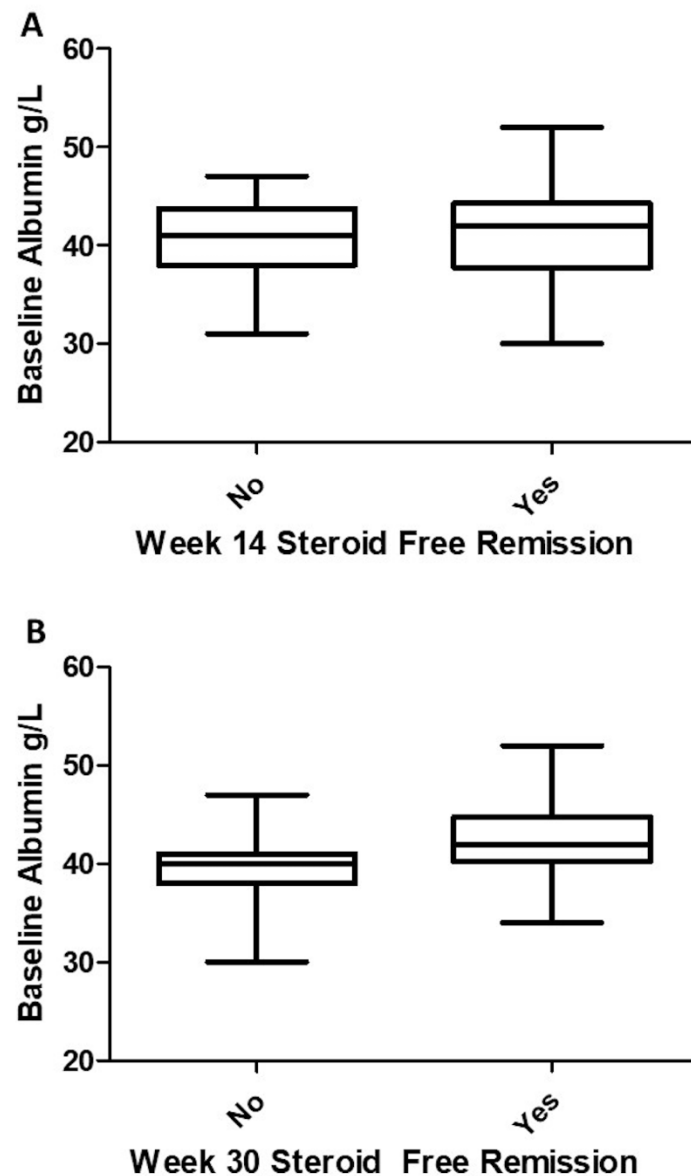


**Figure 2.18: Boxplots Showing the Median, IQR and Range of Baseline Serum Albumin (g/L) in Those With and Without Steroid Free Remission at Week 14 and Week 30.**

No difference in baseline serum albumin was found between those with and without steroid free remission at weeks 14 and 30.

#### 2.4.3.4.5 Vedolizumab Induction and Faecal Calprotectin

Eight of 29 baseline faecal samples had calprotectin levels that were above the upper limit of quantification preventing accurate comparison of the median baseline level with the post induction concentrations. However a non-significant increase in the percentage of patients with calprotectin  $<250\mu\text{g/g}$  was seen following induction (11/24, 46%) compared to baseline (7/29, 24%),  $p=0.09$ .

Only four patients had a normal baseline faecal calprotectin ( $\leq 50\mu\text{g/g}$ ). Patients with a faecal calprotectin  $>50\mu\text{g/g}$  were significantly less likely to be in week 14 SFR (OR 1.4, 95%CI 1.005–1.95,  $p=0.026$ ). However, this was not seen for week 30 SFR,  $p=0.7$ .

In a pilot study of seven individuals with week 2 stool samples available, VDZ was not detected in any faecal sample.

## 2.5 Strengths and Limitations

This was a prospective multicentre study that recruited patients across three Dublin hospitals. The study was well done with consistent protocols. It was a pragmatic study with treatment strategy left to the discretion of the treating physician while coupling high quality clinical research with clinical care. The results of the maintenance and induction studies reflect the real world experience with all its inherent limitations. The prospective design of the induction study allowed for excellent data capture with only two patients lost to follow up. The 41% SFR for patients on maintenance therapy is excellent testament to the standard of clinical practice and effectiveness of the protocols in use in the Dublin centres. As all centres were tertiary referral centres there was an inherent risk for referral bias, however as the centres also provide secondary level care to the local catchment area, the patients recruited reflect the full range of IBD complexity and thus results are generalisable to the IBD population.

There were some limitations. The study cohort consisted of patients with CD and UC. The primary analyses were carried out using their pooled data sets. In the subgroup analysis the smaller

numbers in renders the analysis vulnerable to a type 2 error; a true difference may not have been detected. A larger study would be needed to address this,

A further limitation was that endoscopic endpoints were not available in this study reflecting the real world pressures on the endoscopic service. The inability to detect VDZ in the stool could reflect either a procedural issue or absence of VDZ in the stool. The VDZ drug level assay used in this study, IDKmonitor® Vedolizumab drug level/Anti-VDZ ELISA (Immundiagnostik, Stubenwald-Allee 8a, D-64625 Bensheim) is commercially available, specific and selective for VDZ, and validated for serum measurements but not for stool detection.(342) Although there is good correlation between similar commercial assays (342, 341) comparisons of the results in this study with those of other data sets could be confounded by inter assay. When interpreting our data generated using the IDKmonitor® ELISA, VDZ levels might be slightly underestimated compared with reference assays from the drug manufacturer, Takeda Pharmaceutical Company Limited.(342)

## 2.6 Discussion

The treatment of IBD remains challenging. Despite the increasing range of therapies available, sustained response rates fall short of expectations. Even with newer biologic therapies such as VDZ, a significant proportion of patients fail to respond or, having responded initially, subsequently relapse. These studies were undertaken to help our understanding of the role of VDZ trough concentrations in determining treatment success or failure. We investigated the relationships of VDZ trough levels, in patients on maintenance and those undergoing induction, with treatment outcome as indicated by presence of SFR and persistence on VDZ therapy. We also examined the interplay of VDZ trough concentrations and biomarkers of disease outcome; CRP, serum albumin and faecal calprotectin.

In a snapshot analysis of patients established on maintenance therapy, median duration 10.6 months (46 weeks), 41% were in SFR. No association between serum VDZ trough levels obtained before a routinely scheduled infusion and SFR was found, either for patients with CD or UC, although a trend towards a higher trough VDZ levels in patients with UC in SFR was observed. In a

collaboration with researchers in Mount Sinai Hospital, Toronto, Canada, the data from this cohort formed part of the data set of a larger international study of 94 patients with IBD (40 CD, 54 UC) who were receiving VDZ as maintenance therapy. In the larger study there was also no difference in the median VDZ trough level during maintenance between those achieving clinical remission and those who did not.(343) The lack of clinical utility of maintenance VDZ trough level is a potentially important findings. The consistency of our data with that obtained from heterogenous population in Europe and North America, speaks to its robust status.

Controversy exists in the literature regarding the clinical utility of maintenance VDZ trough levels. A study of 73 patients (30 UC, 43 CD) showed no association between trough level and endoscopic, clinical, or biochemical remission.(344) A larger cross sectional study of 171 patients with CD and UC found no association between VDZ trough levels and mucosal healing.(345) However, in a cross sectional multicentre study examining 258 patients with IBD (UC and CD) undergoing VDZ maintenance therapy, higher VDZ trough levels associated with both clinical and biochemical remission ( $p=0.002$ ) and endoscopic remission ( $p=0.003$ ). Those with VDZ levels  $>11.5\mu\text{g/mL}$  were nearly 2.4 times more likely to be in SFR.(346)

There is also some evidence that higher VDZ levels may be associated with histological remission in UC. A retrospective study of 35 patients on VDZ maintenance therapy found higher median VDZ trough levels in patients in histological remission ( $p=0.02$ ), with a cut off VDZ trough level of  $25\mu\text{g/mL}$  as determined by receiver operator curve analysis, giving an accuracy of 74%, sensitivity 77% and specificity 71%.(347)

A 2019 meta-analysis assessed VDZ trough levels in patients receiving maintenance therapy and found that, based on four studies looking at clinical remission, a trough levels of  $>12\mu\text{g/mL}$  associated with a better therapeutic outcome.(341) When focusing specifically on week 22 results, VDZ trough concentrations  $>13.6\mu\text{g/mL}$  are associated with mucosal healing in patients with CD (71% specificity; 69% sensitivity; AUC, 0.70;  $p=0.018$ ). (341)

Ungar et al., in a prospective observational study reported results based on 129 patients with IBD who required VDZ dose optimisation during maintenance due to non-response, loss of response, or physicians' judgement. There was no statistically significant difference in VDZ trough levels pre-intensification among those reaching clinical remission after dose escalation, compared with those clinically active after the intervention  $p = 0.09$ .(33)

In our study, we did not find evidence to support the clinical utility of trough VDZ monitoring during maintenance therapy. This could possibly reflect a limitation of the cohort size however, the results of the larger collaborative study, of which our data formed a part, also yielded the same result.(18) The median trough concentration in this cohort at  $9.5\mu\text{g/mL}$  fell below that reported by others to be predictive of better outcomes. However the 41% SFR in this study is consistent with, and even exceeds, that generally reported for IBD and is not indicative of sub-standard dosing. Differences in patient populations, disease stage and duration of therapy prior to sampling might all contribute to the disparities in results between this and other studies.

As proposed by Ungar et al.,(348) higher pre-intensification levels may reflect less drug clearance and less severe disease and higher likelihood of subsequent remission, regardless of therapy escalation. In some cases, VDZ trough levels during maintenance therapy may simply act as a marker of disease activity. Thus, the clinical relevance of continued VDZ trough level monitoring during maintenance therapy may be less important than clinical disease assessment.

In the induction cohort, clinical follow-up was excellent with 95% of patients in follow-up at 20 months and 93% at 24 months. Almost two-thirds of patients persisted on VDZ at 12 months dropping to just under half of all patients at 24 months. Consistent with reports in the literature of better VDZ responses in anti-TNF naïve patients,(349-351) in this study in those naïve to anti-TNF VDZ persistence was longer than in those previously treated with anti-TNF (91% at 12 and 73% at 24 months compared with 63% at 12 and 46% at 24 months) although these differences fell short of meeting the threshold for statistical significance.

In the induction cohort 45% percent of patients were in SFR at week 14 and 40% at week 30. The week 6 trough VDZ levels were significantly higher in patients in remission at week 14 ( $p=0.015$ ) and in those in remission at week 30 ( $p=0.029$ ) than in those with persistent disease activity. Using receiver operating curve statistics the value of a week 6 trough level was confirmed and a threshold of  $\geq 15.5\mu\text{g/mL}$  identified a sensitive (82.5-86.5% sensitivity) and moderately specific (65-63.6% specificity) predictor of SFR at week 14 and week 30. Further confirmation of the value of the 6 week trough was evidenced by the significant association of a trough concentration of  $\geq 15.5\mu\text{g/mL}$  with treatment persistence.

In a meta-analysis of real world data that included 10 studies (5CD and 5UC) the 14 week SFR rates were just 25% [95%CI 20–31%] and of 26% [95%CI 20–34%] for CD and UC respectively.(331) Week 6 trough concentrations have been reported to correlate with endoscopic(352, 353), clinical(284, 354, 355) and histologic(356) remission. In the post-hoc analysis of the GEMINI trial, week 6 and week 10 trough levels associated with higher clinical remission rates,(133, 284) and in a systematic review with meta-analysis, a week 6 trough VDZ concentration of  $>20\mu\text{g/mL}$  associated with better clinical outcomes. (341)

In our study VDZ persistence at two years was 46%. Kotze et al. in their cohort, 68% of whom had prior biologic therapy exposure, reported a VDZ persistence rate of 53% at two years.(357) Pudipeddi et al. reported significantly higher persistence at two years, 64%, in those on first line VDZ therapy compared with 54% for patients receiving second line VDZ therapy.(351) In this study and supported by the literature, prior anti-TNF exposure can impact VDZ persistence.(349-351) The slightly lower persistence rate of 46% in our cohort likely reflects the high number of treatment experienced patients and the inclusion of a number of patients on VDZ as a third line regimen.

The week 14 remission rate in this study was higher than might be anticipated from the literature. Although quite a high proportion of patients had previous biologic exposure (77%), the overall disease activity was mild to moderate at time of enrolment with median disease activity scores of seven for CD and four for UC. The local practice at the time of study enrolment of substituting VDZ

for systemic immunosuppressive therapy (e.g., thiopurines) in those stable on treatment if aged 65 years or older or at increased risk of infection could also be a factor in the higher SFR rate found.

The week 30 SFR rate (40%) and VDZ treatment persistence data are also consistent with the published literature (6, 7, 285, 330, 331). The similar SFR rates in the snapshot analysis of maintenance patients (41%) and in the induction cohort at 30 weeks (40%) are noteworthy. They suggest that there may be a stabilisation of response and that there is a patient group capable of sustaining a durable response to VDZ. The overall data confirm the real-world effectiveness of VDZ in IBD. The data also show that up to 60% of patients have a limited response and highlight the need for further research to better characterise the long term responders and to unravel reasons for treatment failure.

A high proportion of our patients had previous exposure to anti-TNF therapy. Recent data for moderate to severe UC indicates that when used as first line therapy VDZ is associated with longer persistence than IFX. However, this persistence advantage is lost when VDZ is used subsequent to IFX.(351) This may explain why 60% of patients had a limited response to VDZ and only 46% continued treatment for more than 24 months. The apparent impact of anti-TNF exposure on VZD persistence, consistent with other reports, suggests that attention to agent positioning might be an important factor to optimise the overall clinical benefit. While prior anti-TNF use can negatively impact VDZ response conversely, it is reported that the response to anti-TNF agents is not affected by prior VDZ therapy.(351) Further studies comparing treatment persistence and response in larger populations of naïve patients, those for whom VDZ is a second line to IFX, and those receiving anti-TNF agents subsequent to VDZ could help inform clinical practice with regard to the optimal sequencing of IBD therapies.

Finding that the week 6 trough concentration is a valuable marker is consistent with data in previous studies. Identifying it as a valuable marker provides a time point suitable for interventions such as dose escalation and could help guide the subsequent treatment course. Failure to confirm this in the subgroup analysis by disease phenotype likely reflects an inherent limitation due to the smaller

numbers involved. The absence of any significant difference in trough concentrations between those with and without SFR at week 2 is likely simply a reflection that it was too early in the course to anticipate an impact from VDZ.

Our study confirms that low week 6 VDZ trough concentrations are associated with a higher chance of treatment failure. A week 6 trough concentrations  $\geq 15.5 \mu\text{g/mL}$  could provide reassuring data for clinicians and, in the clinically appropriate setting, might permit de-intensification of clinical monitoring. Conversely, detection of low trough concentration could identify high risk patients that may benefit from more intense treatment. These data prompt the question as to whether a dose optimisation strategy could further increase rates of SFR?

Our study was an opportunistic real world study that combined CD and UC patients within the same cohort notwithstanding the practice of supplementing induction of CD patients with an additional VDZ infusion at week 10. It is notable that although there was no difference in week 2 VDZ concentrations by disease phenotype, week 6 concentrations were significantly lower in CD than UC,  $p=0.047$ . Our finding that despite this SFR rates at week 14 and 30 were similar is consistent with the findings in a meta analysis of real world studies with week 14 remission rates for CD and UC of 25% and 26% respectively.(331) The additional induction dose received by patients with CD at week 10 could explain why despite the lower concentrations at week six, SFR rates at week 14 and 30 remain comparable however, our study was not designed to address this question.

In the opportunistic post-hoc analysis of the maintenance cohort wherein 27 patients received VDZ every eight weeks and seven patients were receiving infusions every six weeks no significant difference trough concentrations was detected,  $p=0.118$ .

A systematic review that included ten studies reporting dose escalation during maintenance found response rates ranged from 40–73%.(358) No data from control groups continuing therapy without escalation were included. Of the 10 studies included (335, 353, 354, 359-365), none relied on the drug level as the sole indicator for dose escalation. Dreesen et al., reported a significant increase in

the median trough VDZ from 6.8 µg/mL to 13.4 µg/mL, in 17 patients who underwent dose escalation,  $p=0.0002$ .(353)

Willet et al., compared 30 patients who required dose optimisation based on loss or response post induction with 17 remaining in sustained response and found no difference in the VDZ trough concentrations.(354) Ungar et al. in a study of 161 patients with IBD determined that their results did not support pharmacokinetics as the mechanism responsible for loss of response to VDZ, nor a need for higher drug concentrations.(33)

There is equipoise regarding the merits of dose escalation. Our studies were not designed to address that particular issue. While the numbers in the post-hoc analysis are small there is no indication that the shorter interval, based on the physicians discretion, resulted in higher trough levels. Further research with well-designed randomised controlled trials will be needed to provide a definitive answer regarding dose optimisation strategies. Other factors, e.g., disease severity and increase in drug clearance, presence of antidrug antibodies, must be also considered when seeking explanations for treatment failure.

In these cohorts antibodies to VDZ were not detected in any of the patients tested during induction or during maintenance therapy. Similarly, in the expanded international cohort of patients in maintenance therapy, anti-drug antibodies were not detected.(343)

It has been hypothesised the development of anti-VDZ antibodies may contribute to treatment failure. In the GEMINI-1 and GEMINI-2 studies 3.7–4.1% of patients had anti-drug antibodies detected at some time during the study however, only 0.4–1% of patients had detectable anti-drug antibodies present in more than one consecutive sample.(313, 314) Real world studies have consistently shows very low rates (<1%) of anti-VDZ antibodies during treatment(345, 354) with one study finding them present in a single sample out of 928 samples.(353) That VDZ is not immunogenic possibly explains why concomitant immunomodulator therapy does not improve outcomes when added to VDZ (323, 346, 347) unlike when they are used in combination with

IFX.(212) Suppression of anti-IFX antibodies by immunomodulators, in essence releasing IFX from antibody binding and neutralisation, is thought to account for the better outcomes when immunomodulators are given together with IFX. The same effect has not been found for VDZ.(366)

It is thought that concomitant immunomodulator therapy might prevent or reduce development of anti-drug antibodies, inhibiting drug inactivation through antibody binding, and thus might be associated with higher trough levels of the concomitant biologic medication. The absence of detectable antibodies to VDZ in any of the patients we tested, suggests that antibody binding as a cause of drug inactivation is not a significant issue for VDZ.

As a less immunogenic agent than some of the alternate biologic therapies, VDZ represents an important therapeutic option for patients with IBD.

CRP, serum albumin and faecal calprotectin are all considered biomarkers of disease activity and thus might be subject to alteration during VDZ therapy and/or serve as indicators of likely VDZ response. In the maintenance study, no correlations between trough VDZ concentrations and concomitant CRP, serum albumin, or faecal calprotectin were found.

In the induction study, where 60% patients had a baseline CRP<5mg/L no evident impact of VDZ induction on CRP levels was detected. However, a low baseline CRP (<5mg/L) associated with higher week 6 trough VDZ concentrations and higher VDZ trough levels associated with lower CRP levels at weeks 6 and 10-14. In keeping with these findings, those with a lower baseline CRP (<5mg/L) were more likely to be in SFR at week 14. Although the trend suggests that this may also pertain at week 14 the difference in baseline CRP between those in SFR at week 30 and those with persistent disease activity just fell short of achieving significance,  $p=0.055$ .

In the cross-sectional snapshot of patients on maintenance therapy no correlation between serum albumin and VDZ trough concentrations was found. In the induction cohort, there was a significant positive correlation between serum albumin and VDZ trough levels. Baseline serum albumin

however did not differentiate between those likely and unlikely to be in SFR at either week 14 or week 30.

A post-hoc analysis of the VDZ GEMINI study showed that lower baseline CRP associated with treatment response in CD.(284) Other retrospective observational studies have also shown that a higher CRP is more likely associated with treatment failure.(285, 286, 347, 353) It has been reported that patients with a baseline CRP >20mg/ml in one study (278) and >5mg/ml in another (338) are less likely to achieve clinical remission at week 14 than those with baseline CRP below the aforementioned cut offs. Similarly lower CRP has been associated with higher concentrations of anti-TNF trough levels in IBD patients.(367) In other studies lower CRP has been shown to be associated with higher VDZ levels for both UC and CD.(345, 353) However, In patients undergoing dose optimisation during maintenance VDZ therapy CRP was not significantly different responders and non-responders.(324)

Low serum albumin levels is considered a marker of disease activity, thought to be due to a protein losing enteropathy that can be present in severe IBD.(287) Low serum albumin is linked poor outcomes in patients presenting with acute colitis.(288) Lower albumin is also associated with lower trough drug levels across a range of monoclonal antibodies used to treat oncological conditions,(289) and with IFX in treatment of IBD.(290, 291) This may be because a low albumin is a marker of disease activity and may reflect increased drug metabolism. For VDZ, higher serum albumin has been associated with higher trough levels in maintenance therapy.(344, 368, 369) The association of albumin with drug levels during induction has been used to develop a pharmacokinetic–pharmacodynamic model targeting endoscopic remission in CD that is awaiting prospective study.(370)

Disease severity is an important factor that could influence response to VDZ. CRP, albumin, and faecal calprotectin are all biomarkers of disease severity. Study of the interactions of VDZ with these biomarkers was thus undertaken. In patients already well established on therapy of varying

duration, the maintenance cohort, no correlations between CRP, serum albumin or faecal calprotectin with VDZ trough levels were found.

The results of the induction studies show that while CRP is not directly influenced by VDZ administration in our study, it is a marker of disease activity; lower baseline levels associated with SFR and higher levels associated with lower VDZ trough concentrations. The findings presented suggest that baseline CRP could be used as a predictive marker to identify patients who may fail VDZ. As higher CRP levels consistently associated with lower VDZ trough levels across different time points during induction, there is potential to use it as a surrogate marker for VDZ trough levels and thus to identify patients who may benefit from dose optimisation following induction. The lack of correlation identified in the maintenance may reflect confounding inherent in the cross-sectional nature of the study and the limitations of the patient population; the limited number with data available (n=21); variability in treatment duration; and that patients were well established on therapy almost two-thirds of whom had a CRP<5mg/L.

Our study did not show a correlation between albumin levels and VDZ drug levels during maintenance phase, this may be due to the comparatively low numbers of patients for which we had data (n=21) and that all patients had a serum albumin within the normal range (35–45g/L). In the induction cohort, however, with larger numbers and a greater range of serum albumin values (30–52g/L) there was significant consistent correlation between serum albumin and VDZ trough levels during induction period. Serum albumin was associated with trough VDZ at the time of the second, third and fourth infusions.

## 2.7 Conclusion

This prospective multicentre study found a significant association between the week 6 pre infusion VDZ concentration and treatment remission status at week 14 and week 30. Higher VDZ levels at week 6 are also associated with persistence of VDZ therapy. Measurement of week 6 VDZ pre-infusion concentration can provide a valuable indication as to the likely success of VDZ treatment or need to consider dose optimisation. Patients with CD had significantly lower VDZ concentrations

at week 6 despite which SFR rates at week 14 and 30 were comparable to those of patients with UC. This could reflect the importance of the additional week 10 VDZ infusion for patients with CD.

Serum levels of CRP and albumin correlate with VDZ levels during induction and could potentially be used as surrogate markers of VDZ trough levels and help identify suitable candidates for VDZ dose optimisation. However the benefit of and the most effective strategy for dose optimisation of VDZ remains to be confirmed in prospective trials.

## 2.8 Future Directions

Further prospective randomised controlled trials of the effectiveness of VDZ dose optimisation and positioning as first or second line therapy are required, as robust evidence to guide clinical practice is lacking. The data in our studies agree with the published literature that low week 6 VDZ levels are associated with increased likelihood of treatment failure. However, whether the low drug levels simply reflects inadequate dosing or act as a surrogate marker of disease severity resulting from increased drug metabolism / increased drug loss, is uncertain. The findings suggest that the  $\alpha_4\beta_7$  pathway is in fact often saturated even at very low concentrations of VDZ so patient characteristics may play an important role in treatment response/failure. To test this a prospective randomised controlled trial comparing proactive dose escalation in induction and maintenance addressing response in differing phenotypic groups versus best medical management would be enlightening.

Our study has shown that VDZ drug levels correlated with albumin and CRP. As has shown to be efficacious in IFX therapy for UC the development of a VDZ dashboard, taking into account biomarkers of disease activity and drug clearance such as weight, CRP, albumin, and trough levels could allow for personalised VDZ dosing. Prospectively comparing dashboard dosed VDZ against best medical therapy in a RCT as was done for IFX could clarify whether or not dose optimisation of VDZ should become a standard of care in IBD.

## Chapter 3.0: Investigation of Potential Serum Biomarkers of Response to Vedolizumab in Patients with Inflammatory Bowel Disease.

### 3.1: Introduction

Vedolizumab (VDZ), a humanised monoclonal antibody, specifically recognizes the  $\alpha_4\beta_7$  integrin and selectively blocks gut lymphocyte trafficking, thus exerting an anti-inflammatory effect on intestinal tissue.(6, 7) Integrins are cell surface glycoprotein receptors that are postulated to play a role in the pathogenesis of inflammatory bowel disease (IBD).(371) The  $\alpha_4\beta_7$  integrin is variably expressed on circulating B and T lymphocytes,(372) NK cells,(373) mast cells,(374), basophils and monocytes.(375) It binds with the mucosal addressin cell adhesion molecule 1 (MAdCAM-1), an IgG family adhesion molecule, expressed on and confined to the intestinal vasculature.(372) It directs trans-endothelial lymphocyte migration to the gut mucosal surfaces thus contributing to the inflammatory process in IBD. This binding can also lead to co-stimulation of T cells,(376) similar to their stimulation by the CD 28 receptor,(377) leading to T cell proliferation and cell migration to the gut. Recent papers have postulated that the  $\alpha_4\beta_7$ -MAdCAM-1 interaction leading to lymphocyte stimulation and migration may not be its only role in gut inflammation and the  $\alpha_4\beta_7$  integrin may be involved in additional mechanisms other than promoting lymphocyte migration.

In a phase III randomised controlled trial (the GEMINI study), VDZ was demonstrated to be effective both as induction and maintenance therapy in Crohn's disease (CD)(6) and ulcerative colitis (UC).(7) It is one of several newer biologic therapies approved for IBD treatment. However, a significant proportion of patients do not respond to VDZ treatment. Consistent with results of the GEMINI study(6, 7) a metanalysis of real world studies found that only 30% patients with CD and 32% those with UC were in remission at week 14 of VDZ therapy.(331) However, long term follow up of Gemini participants showed that up to 28% of those with CD and 33% with UC were in remission at week 400 (285), indicating that there is a subset of individuals who have an enduring response to VDZ. However, as yet there is no selection tool or biomarker assay to select those that might respond to VDZ.

Vedolizumab's postulated mechanism of action is through blocking lymphocyte migration to gut.(6, 7) Recent papers however question VDZ's ability to inhibit lymphocyte trafficking in vivo.(377, 378) Immunohistological staining of colonic specimens before and during VDZ treatment showed no change in CD4+ T cells and regulatory T cells populations.(377)

Cytokines encompass a wide range of small proteins that are actively secreted by immune cells and are critical mediators of cell-to-cell signalling. Chemokines, a subset of the larger cytokine family, are small peptides and mainly act as chemotactic factors for inflammatory cells. Research as to how VDZ impacts inflammatory markers .i.e., the cytokines, chemokines and growth factors, is required to gain more detailed insight to VDZ's mechanisms of action. Such study could also possibly identify a biomarker or biomarkers that that are predictive of VDZ response and have significant benefit in enabling targeted patient selection for treatment.

### 3.2: Specific Aim

The aim of this study was to assess the impact of VDZ induction on a panel of serum inflammatory markers in patients with IBD and to determine whether the baseline concentrations of any might be predictive of a therapeutic response at weeks 14 and 30.

### 3.3: Methods

#### 3.3.1 Ethics and Consent

This project was approved by the Joint St James Hospital and Tallaght University Hospital Research and Ethics Committee and the St Vincent's Hospital Research and Ethics Committee(approved 18 September 2017);. Written informed consent was obtained from all patients prior to enrolment. SJH/AMNCH Research Ethics Committee Secretariat Reference Number 2017-04 List 12.

#### 3.3.2 Study Population

This was a prospective multi-centre cohort study of IBD patients recruited from three Irish academic medical centres. Enrolment criteria included patients identified by members of the Investigator Network for Inflammatory Bowel Disease Therapy in Ireland (INITiative) with an established diagnosis of CD or UC and who were scheduled to commence VDZ induction therapy. Patients were

ineligible if they were aged under 18 years, unable to give consent, had previous VDZ therapy, or were pregnant or breastfeeding.

Baseline demographics and clinical data were obtained by patient questionnaire complemented by medical record review at the time of enrolment. Variables collected included: age, weight, gender, disease duration, 5-aminosalicylate (5-ASA) use, concurrent immunomodulator use (thiopurines/methotrexate), previous biologic therapy exposure (IFX, adalimumab, ustekinumab, golimumab), previous small molecule inhibitors (tofacitinib) use, and current glucocorticoid therapy. Disease extent and location was classified using the Montreal classification.<sup>(336)</sup> Where available, pre-VDZ endoscopies were reviewed and disease severity assessed based on the Harvey Bradshaw Index (HBI) for patients with CD and the partial Mayo sub-score for patients with UC.

### 3.3.3 Vedolizumab Administration

Vedolizumab was administered according to the standard protocol with all patients receiving induction therapy with 300mg infusions at weeks 0, 2 and 6. Patients with CD received an additional 300mg infusion at week 10. Following induction all patients received a VDZ infusion every eight weeks as maintenance therapy. Patients were monitored throughout the induction period (10-14 weeks) and while on maintenance therapy for up to 41 months.

### 3.3.4 Sample Collection and Analysis

Serum samples were obtained immediately before VDZ infusions at baseline i.e., week 0; and week 6 and 14. Complete blood count, biochemistries (liver and renal profile), serum albumin, and C-reactive protein (CRP) were measured at week 0 and 14. The baseline and week 6 samples for multiplex cytokine screening were stored at -80°C and batched for later analysis. A VDZ level greater than 12µg/mL was considered therapeutic.<sup>(341)</sup> Serum samples were obtained for VDZ levels prior to each infusion and before the fourth VDZ infusion for anti-VDZ antibody. Samples were tested using commercially available ELISA test kits (IDKmonitor® Vedolizumab drug level/Anti-VDZ ELISA, Immundiagnostik Stubenwald-Allee 8a, D-64625 Bensheim). Samples for faecal calprotectin detection were collected the night before or morning of, the first, third, and fourth VDZ infusions.

### 3.3.5 Endpoints and Definitions

Crohn's Disease severity was measured using the HBI with a score of 8 or higher indicating severe disease.(337) UC disease severity was assigned a score on a scale of 0 to 9, using the partial Mayo sub-score. Higher scores, 7 to 9, indicate more severe disease.(339) Disease severity was assessed at baseline and weeks 14, 30 and 54.

Clinical remission was defined as a HBI score of less than or equal to five for CD, a partial Mayo sub-score of less than or equal to one for UC, no requirement for glucocorticoid therapy, and continuing treatment with VDZ.

The primary endpoint was Steroid Free Remission (SFR) at week 14. Secondary endpoints included SFR at week 30 and time to discontinuation of VDZ therapy.

The reasons for VDZ discontinuation were categorised as follows; primary nonresponse was defined as drug discontinuation because of lack of response within three months of therapy initiation, secondary loss of response was defined as drug discontinuation because of lack of response after at least three months had elapsed from the initiation of VDZ therapy.

### 3.3.6 Biomarker Assays

Pre infusion serum samples were run using a V-PLEX Human Biomarker 54-plex enzyme-linked immunosorbent assay (ELISA) kit (Meso Scale Discovery, Rockville, MD, USA.)(379) that included a vascular injury panel, cytokine panel, chemokine panels 1 and 2, angiogenesis panel, pro-inflammatory panel, and TH17 panel which in total had the capability to detect 54 inflammatory markers.

CRP, eotaxin, eotaxin-3, FGF(basic), FLT-1, GM-CSF, ICAM-1, IFN- $\gamma$ , IL-10, IL-12/IL-23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-1 $\alpha$ , IL-1 $\beta$ , IL-2, IL-21, IL-22, IL-23, IL-27, IL-3, IL-31, IL-4, IL-5, IL-6, IL-7, IL-8, IL-8 (HA), IL-9, IP-10, MCP-1, MCP-4, MDC, MIP-1 $\alpha$ , MIP-1 $\beta$ , MIP-3 $\alpha$ , PIGF, SAA, TARC, Tie-2, TNF- $\alpha$ , TNF- $\beta$ , TSLP, VCAM-1, VEGF-A, VEGF-C and VEGF-D were measured. All assays were run according to the manufacturer's guidelines and the results expressed in pg/mL. Serum samples were diluted, 1:2 for the angiogenesis, proinflammatory, and

cytokine panels 1 and 2. A 1:4 dilution was used for the TH17 panel and 1:1,000 dilution for the vascular injury panel.

### 3.3.7 Statistical Analysis

Descriptive statistics were applied to the baseline demographics with continuous variables presented with median and range and categorical variables summarised as percentages. The Mann Whitney U test was used for categorical and continuous independent data. Paired continuous data was interpreted using the Wilcoxon sign-rank test.

Pre-specified analyses included the association of week 14 and week 30 SFR with baseline concentrations of the inflammatory markers and the association of VDZ with SFR and with the marker concentrations. The response of individual inflammatory markers to VDZ induction were assessed by comparing their concentrations at week 0 with week 6. Kaplan–Meier survival curves were constructed for time-based analyses and differences between groups evaluated using the log-rank test.

In our secondary analyses, we applied a false discovery rate (FDR) control using the Benjamini–Hochberg procedure for multiple test correction with the FDR assigned being 5%. As per standard protocol, the largest p value which was less than the Benjamini–Hochberg critical value was identified. All comparisons with a p value less than or equal to this threshold p value were considered significant following FDR control. All statistical analyses were performed using SPSS (version 26.1.2; IBM, NY, USA). Graphs were constructed using GraphPad Prism (version 5.01 : GraphPad Software Inc.).

## 3.4 Results

### 3.4.1 Patient and Disease Characteristics

Forty-three patients were recruited from three hospitals as part of the VDZ induction study (see Chapter 2) with the first enrolment on 15 May 2017 and the last patients enrolled on 20 February 2019. Four patients were excluded from analysis; one withdrew consent, one required colectomy within 48 hours of the initial infusion, two had absent baseline serum samples. (**Figure 3.1**) The

demographics and clinical characteristics of 39 evaluable patients are listed (**Table 3.1**). The median age at time of first infusion was 52 years with almost equal numbers of male and female patients participating. In this study patients with CD slightly outnumbered those with UC. Crohn's disease severity was mild to moderate and included ileocolonic and more localised ileal or colonic involvement. Of patients with UC, 36% had extensive colitis, 64% had left sided colitis and none had proctitis. Ulcerative colitis was of mild to moderate severity overall with a mean baseline partial Mayo sub-score of four. All patients had failed first line IBD therapy, over two thirds of whom had prior biologic exposure with anti-TNF agents indicating relatively refractory disease in this cohort. No patient had prior exposure to JAK-STAT inhibitors.

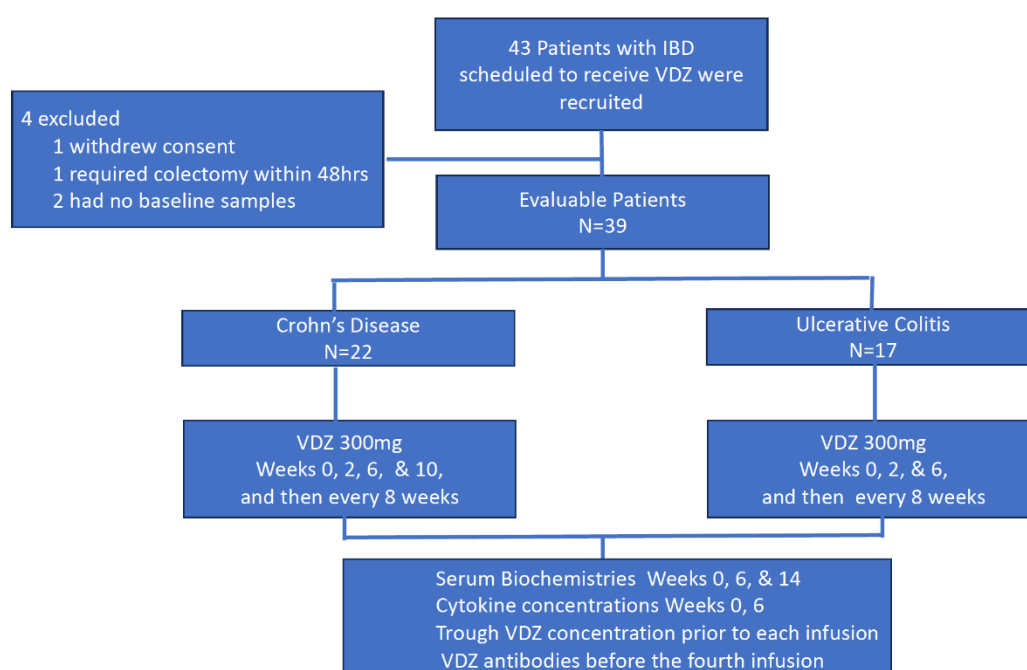


**Figure 3.1. Serum Biomarker Study Flow Diagram**

Forty-three patients with Inflammatory Bowel Disease were recruited over a 20 month period. Following exclusion of four patients, data analysis was based on 39 patients, 22 with CD and 17 with UC

|                                                                                                         | Evaluable Patients<br>N 39                   |
|---------------------------------------------------------------------------------------------------------|----------------------------------------------|
| <b>Age in years</b><br><b>Median [range]</b>                                                            | 50.4 [18.2–75.8]                             |
| <b>Gender</b><br>Female<br>Male                                                                         | 19 (49%)<br>20 (51%)                         |
| <b>Disease Duration (years)</b><br><b>Median [range]</b>                                                | 13.4 [0.4–40.8].                             |
| <b>Crohn's Disease (N22)</b>                                                                            | 22 (56%)                                     |
| <i>Disease extent</i><br>Ileal<br>Colonic<br>Ileocolonic                                                | 6 (27%)<br>4 (18%)<br>12 (55%)               |
| <i>Disease behaviour</i><br>Non-Stricturing Non-Penetrating<br>Stricturing<br>Penetrating               | 10 (46%)<br>8 (36%)<br>4 (18%)               |
| <i>Disease score</i><br>Harvey Bradshaw Index                                                           | 7 [1–20]                                     |
| <b>Ulcerative colitis (N17)</b>                                                                         | 17 (44%)                                     |
| <i>Disease extent</i><br>Proctitis<br>Left-sided colitis<br>Extensive colitis                           | 0<br>6 (36%)<br>11 (64%)                     |
| <i>Disease score</i><br>Partial Mayo Subscore                                                           | 4 [0–9]                                      |
| <b>Faecal Calprotectin (µg/g)</b>                                                                       | 740 [16.4–1250]                              |
| <b>C-Reactive Protein (mg/L)</b>                                                                        | 3.5 [1–43]                                   |
| <b>Albumin (g/L)</b>                                                                                    | 41 [30–52]                                   |
| <b>5-Aminosalicylates</b>                                                                               | 18 (46%)                                     |
| <b>Glucocorticoids</b>                                                                                  | 8 (20%)                                      |
| <b>Immunomodulators</b><br>Thiopurines<br>Methotrexate                                                  | 9 (22.5%)<br>8 (20%)<br>1 (2.5%)             |
| <b>Prior Biologic Therapy</b><br>Naïve<br>≥1 previous agent<br>≥2 previous agents<br>≥3 previous agents | 11 (28%)<br>28 (72%)<br>20 (51%)<br>10 (25%) |

Categorical variables are presented as percentages; in some instances the sum of the percentages exceed 100% due to rounding. Continuous variables are presented as median and range

**Table 3.1 Demographics and Clinical Characteristics of 39 patients with Inflammatory Bowel Disease at Time of Vedolizumab initiation**

### 3.4.2 Patient Outcomes

The median follow-up of patients was 17.8 months [range 0.5-41 months]. By week 14, 17/39 (44%) patients were in steroid free remission (SFR), five of whom were no longer in remission when assessed at week 30. An additional three patients achieved SFR by week 30. Thus, a total of 15 (38%) of patients were in SFR by week 30.

Trough VDZ concentrations at week 2 and week 6 were 25.1mcg/mL [range 0–56mcg/mL] and 20.2mcg/mL [range 0–66mcg/mL] respectively. At the time of the fourth infusion anti-VDZ antibodies were undetectable in all patients. Week 14 faecal calprotectin measurements, available on 25 patients, were indicative of biochemical remission in eight (32%) patients.

At week 30, thirty patients continued to receive VDZ. Nine patients (23%) had discontinued VDZ treatment of whom three had required colectomy within the 30 weeks. Over the course of the study, a total of 23 (58%) patients discontinued VDZ. The median [range] time to VDZ discontinuation was 9.3 months [range 0.5–38.7 months]. Reasons for discontinuation included primary nonresponse, 5 (22%), secondary loss of response, 10 (43%), intolerance or adverse event, 5 (22%), and reasons not stated, 3 (13%). Adverse events included three patients with joint pain, one with deteriorating renal function, and one with recurrent sinusitis.

### 3.4.3 Association of Baseline (Week 0) Concentrations of 54 Inflammatory Markers with Week 14 and Week 30 Steroid Free Remission

Baseline concentrations (week 0) of 54 inflammatory markers, median and [IQR], in patients with SFR at week 14 were compared to those in patients who, at week 14, had persistent disease activity (**Table 3.2**). In univariate analysis of the 54 markers, the baseline concentrations of four; VEGF-C, IL-7, TNF-  $\beta$ , and IL-22, were associated with week 14 SFR (**Figure 3.2**). None of the inflammatory markers that were nominally associated with week 14 SFR remained significant when assessed at week 30. However, the baseline concentrations of four other inflammatory markers; IL-4, MDC, IL-10, and MCP- 4, were associated with week 30 SFR (**Figure 3.3**). When a false discovery rate correction for multiplicity were applied neither differences at week 14 nor those at week 30 reached the threshold for significance at either week 14 or week 30. There was no significant

evidence of association of the baseline concentrations of any of the remaining 50 analytes with either week 14 or week 30 disease status.

| Baseline (Week 0) Concentrations of 54 Inflammatory Markers (pg/mL, Median [IQR]) |                            |                    |                        |                   |         |                            |                    |                        |                    |         |
|-----------------------------------------------------------------------------------|----------------------------|--------------------|------------------------|-------------------|---------|----------------------------|--------------------|------------------------|--------------------|---------|
|                                                                                   | Disease Status at 14 weeks |                    |                        |                   |         | Disease Status at 30 weeks |                    |                        |                    |         |
|                                                                                   | Persistent disease         |                    | Steroid Free Remission |                   | P Value | Persistent disease         |                    | Steroid Free Remission |                    | P Value |
| Angiogenesis Panel                                                                |                            |                    |                        |                   |         |                            |                    |                        |                    |         |
| bFGF                                                                              | 5.89                       | [2.33-8.20]        | 6.33                   | [3.26-14.44]      | NS      | 6.11                       | [2.07-9.22]        | 6.02                   | [3.61-14.21]       | NS      |
| Flt-1                                                                             | 98.61                      | [74.82-123.21]     | 95.13                  | [78.46-111.75]    | NS      | 99.56                      | [74.06-119.67]     | 93.70                  | [82.05-17.7]       | NS      |
| PlGF                                                                              | 4.09                       | [3.37-5.05]        | 3.58                   | [3.34-4.40]       | NS      | 4.07                       | [3.31-5.04]        | 3.58                   | [3.40-4.35]        | NS      |
| Tie-2                                                                             | 1750.65                    | [1632.10-2122.52]  | 1977.75                | [1742.86-2277.49] | NS      | 1839.43                    | [1634.67-2152.27]  | 1925.18                | [1673.51-2253.23]  | NS      |
| VEGF                                                                              | 275.06                     | [167.90-329.63]    | 213.97                 | [145.76-361.86]   | NS      | 215.42                     | [156.45-327.58]    | 281.37                 | [173.33-363.49]    | NS      |
| VEGF-C                                                                            | 135.95                     | [88.65-170.39]     | 103.43                 | [79.73-115.00]    | P=0.042 | 121.32                     | [68.74-150.49]     | 111.91                 | [95.43-122.34]     | NS      |
| VEGF-D                                                                            | 915.93                     | [722.61-1188.92]   | 927.20                 | [837.71-1083.84]  | NS      | 913.73                     | [717.52-1161.09]   | 927.20                 | [811.76-1061.03]   | NS      |
| Chemokine Panel 1                                                                 |                            |                    |                        |                   |         |                            |                    |                        |                    |         |
| Eotaxin                                                                           | 91.32                      | [62.04-126.76]     | 80.481                 | [70.91-107.06]    | NS      | 95.18                      | [67.93-131.29]     | 80.19                  | [72.05-102.79]     | NS      |
| Eotaxin-3                                                                         | 1.53                       | [1.16-2.88]        | 1.385                  | [0.94-2.37]       | NS      | 1.33                       | [1.13-2.81]        | 1.46                   | [0.98-2.77]        | NS      |
| IL-8                                                                              | 27.13                      | [11.36-47.71]      | 32.871                 | [10.61-63.07]     | NS      | 26.67                      | [16.09-49.73]      | 32.87                  | [9.81-52.66]       | NS      |
| IP-10                                                                             | 49.34                      | [35.06-68.09]      | 80.754                 | [41.73-104.21]    | NS      | 57.32                      | [34.26-74.00]      | 62.86                  | [40.05-111.24]     | NS      |
| MCP-1                                                                             | 66.41                      | [51.64-77.65]      | 64.08                  | [49.85-83.26]     | NS      | 60.10                      | [47.33-74.33]      | 70.78                  | [63.49-102.72]     | NS      |
| MCP-4                                                                             | 29.09                      | [20.95-41.60]      | 28.42                  | [21.41-50.28]     | NS      | 26.08                      | [15.92-37.81]      | 34.47                  | [25.57-51.30]      | P=0.028 |
| MDC                                                                               | 304.12                     | [174.21-350.64]    | 336.97                 | [251.15-364.76]   | NS      | 295.36                     | [153.91-333.58]    | 356.50                 | [302.55-375.52]    | P=0.026 |
| MIP-1α                                                                            | 4.52                       | [3.25-5.48]        | 4.57                   | [3.89-6.56]       | NS      | 4.50                       | [3.22-5.60]        | 4.70                   | [3.96-6.59]        | NS      |
| MIP-1β                                                                            | 28.21                      | [20.01-37.08]      | 32.70                  | [23.09-36.66]     | NS      | 27.84                      | [16.95-37.64]      | 34.34                  | [26.24-37.08]      | NS      |
| TARC                                                                              | 61.18                      | [44.05-92.94]      | 60.89                  | [46.03-96.39]     | NS      | 62.48                      | [49.00-92.75]      | 49.57                  | [39.67-102.85]     | NS      |
| Chemokine Panel 2                                                                 |                            |                    |                        |                   |         |                            |                    |                        |                    |         |
| IL-17A/F                                                                          | 0.70                       | [0.31-1.53]        | 0.44                   | [0.26-1.32]       | NS      | 0.49                       | [0.17-1.33]        | 0.80                   | [0.42-1.43]        | NS      |
| IL-17B                                                                            | 0.04                       | [0 -0.75]          | 0                      | [0 -0.19]         | NS      | 0.00                       | [0.00-0.47]        | 0.07                   | [0.00-0.53]        | NS      |
| IL-17C                                                                            | 0                          | 0                  | 0                      | 0                 | NS      | 0                          | 0                  | 0                      | 0                  | NS      |
| IL-17D                                                                            | 2.97                       | [1.20-4.45]        | 1.63                   | [0.30-3.50]       | NS      | 2.60                       | [0.42-4.41]        | 1.89                   | [1.46-4.14]        | NS      |
| IL-1RA                                                                            | 104.89                     | [87.18-164.08]     | 100.62                 | [70.93-119.88]    | NS      | 104.89                     | [82.34-167.48]     | 100.62                 | [76.72-119.02]     | NS      |
| IL-3                                                                              | 0                          | 0                  | 0                      | 0                 | NS      | 0                          | 0                  | 0                      | 0                  | NS      |
| IL-9                                                                              | 0                          | 0                  | 0                      | 0                 | NS      | 0                          | 0                  | 0                      | 0                  | NS      |
| TSLP                                                                              | 0.29                       | [0.19-0.50]        | 0.23                   | [0.14-0.38]       | NS      | 0.29                       | [0.20-0.49]        | 0.23                   | [0.14-0.37]        | NS      |
| Vascular Injury Panel 1                                                           |                            |                    |                        |                   |         |                            |                    |                        |                    |         |
| CRP                                                                               | 7092.67                    | [2048.05-22544.03] | 3823.26                | [2758.16-5441.57] | NS      | 6629.12                    | [1750.92-2364.63]  | 3708.08                | [2815.81-6146.77]  | NS      |
| ICAM-1                                                                            | 629.31                     | [494.13-695.65]    | 560.70                 | [475.80-695.06]   | NS      | 629.31                     | [489.08-699.04]    | 560.70                 | [472.76-689.55]    | NS      |
| SAA                                                                               | 13070.75                   | [2511.91-45884.87] | 5193.49                | [2021.82-5081.21] | NS      | 15247.93                   | [2832.61-44825.49] | 4396.91                | [1923.56-10394.40] | NS      |
| VCAM-1                                                                            | 595.59                     | [507.91-693.42]    | 573.28                 | [502.39-618.07]   | NS      | 600.74                     | [551.56-719.63]    | 563.97                 | [486.74-620.70]    | NS      |

| Baseline Concentrations of 54 Inflammatory Markers (pg/mL), Median [IQR] |                            |                 |                        |                 |         |                            |                 |                        |                  |         |
|--------------------------------------------------------------------------|----------------------------|-----------------|------------------------|-----------------|---------|----------------------------|-----------------|------------------------|------------------|---------|
|                                                                          | Disease Status at 14 weeks |                 |                        |                 |         | Disease Status at 30 weeks |                 |                        |                  |         |
|                                                                          | Persistent disease         |                 | Steroid Free Remission |                 | P Value | Persistent disease         |                 | Steroid Free Remission |                  | P Value |
| Cytokine Panel 1                                                         |                            |                 |                        |                 |         |                            |                 |                        |                  |         |
| GM-CSF                                                                   | 0                          | [0-0.02]        | 0                      | [0-0.01]        | NS      | 0.00                       | [0.00-0.01]     | 0.00                   | [0.00-0.02]      | NS      |
| L-12/1L 23P4                                                             | 70.50                      | [31.67-204.03]  | 68.56                  | [41.31-95.09]   | NS      | 66.72                      | [30.81-174.23]  | 73.63                  | [41.56-167.34]   | NS      |
| IL-15                                                                    | 1.48                       | [1.27-2.17]     | 1.348                  | [1.09-1.50]     | NS      | 1.39                       | [1.23-2.08]     | 1.49                   | [1.07 - 1.65]    | NS      |
| IL-16                                                                    | 103.28                     | [69.83-145.42]  | 79.58                  | [61.35-135.69]  | NS      | 98.42                      | [64.85-141.97]  | 91.85                  | [62.03 - 139.63] | NS      |
| IL-17A                                                                   | 3.60                       | [2.63-4.77]     | 2.58                   | [2.04-4.66]     | NS      | 3.25                       | [2.24-4.91]     | 3.09                   | [2.50 - 4.38]    | NS      |
| IL-1α                                                                    | 0                          | [0-0.13]        | 0                      |                 | NS      | 0.00                       | [0.00-0.00]     | 0.00                   | [0.00 - 0.66]    | NS      |
| IL-5                                                                     | 0                          | 0               | 0                      | 0               | NS      | 0                          | 0               | 0                      | 0                | NS      |
| IL-7                                                                     | 13.0                       | [8.36-18.51]    | 10.54                  | [6.26-12.10]    | P=0.045 | 11.35                      | [7.27-16.85]    | 10.92                  | [7.70-13.09]     | NS      |
| TNF-β                                                                    | 0.09                       | [0.03-0.11]     | 0.14                   | [0.10-0.18]     | P=0.003 | 0.10                       | [0.07-0.13]     | 0.13                   | [0.08 - 0.19]    | NS      |
| Proinflammatory Cytokine Panel                                           |                            |                 |                        |                 |         |                            |                 |                        |                  |         |
| IFN-γ                                                                    | 2.53                       | [1.08-7.79]     | 3.47                   | [2.18-6.01]     | NS      | 2.52                       | [1.15-7.50]     | 3.47                   | [1.68-5.40]      | NS      |
| IL-10                                                                    | 0.32                       | [0.23-0.59]     | 0.26                   | [0.22-0.43]     | NS      | 0.36                       | [0.24-0.63]     | 0.22                   | [0.18-0.31]      | P=0.03  |
| IL-12p70                                                                 | 0.13                       | [0.07-0.22]     | 0.09                   | [0.06-0.17]     | NS      | 0.11                       | [0.06-0.19]     | 0.12                   | [0.07-0.19]      | NS      |
| IL-13                                                                    | 0.35                       | [0.28-0.50]     | 0.36                   | [0.21-0.47]     | NS      | 0.33                       | [0.24-0.45]     | 0.42                   | [0.20-0.62]      | NS      |
| IL-1β                                                                    | 0.02                       | [0-0.10]        | 0.04                   | [0-0.09]        | NS      | 0.02                       | [0.00-0.09]     | 0.04                   | [0.00-0.09]      | NS      |
| IL-2                                                                     | 0.07                       | [0.05-0.1]6     | 0.09                   | [0.04-0.12]     | NS      | 0.07                       | [0.05-0.11]     | 0.11                   | [0.05-0.15]      | NS      |
| IL-4                                                                     | 0.01                       | [0.01-0.02]     | 0.01                   | [0.01-0.03]     | NS      | 0.01                       | [0.01-0.02]     | 0.02                   | [0.01-0.03]      | P=0.011 |
| IL-6                                                                     | 0.82                       | [0.47-1.56]     | 0.63                   | [0.52-0.74]     | NS      | 0.73                       | [0.44-1.29]     | 0.70                   | [0.60-0.81]      | NS      |
| IL-8                                                                     | 12.45                      | [9.45-17.32]    | 11.10                  | [8.07-24.65]    | NS      | 12.15                      | [9.03-18.69]    | 13.80                  | [9.19-27.15]     | NS      |
| TNF-α                                                                    | 1.53                       | [1.27-2.23]     | 1.99                   | [1.42-5.98]     | NS      | 1.47                       | [1.30-2.20]     | 2.18                   | [1.56-4.64]      | NS      |
| TH 17 Panel                                                              |                            |                 |                        |                 |         |                            |                 |                        |                  |         |
| IL-17A <sub>GenB</sub>                                                   | 0.26                       | [0-0.68]        | 0.09                   | [0-0.46]        | NS      | 0.21                       | [0.00-0.68]     | 0.09                   | [0.00-0.52]      | NS      |
| IL-21                                                                    | 0.00                       | [0- 0.11]       | 0                      | [0 -0.01]       | NS      | 0.00                       | [0.00-0.07]     | 0.00                   | [0.00-0.05]      | NS      |
| IL-22                                                                    | 0.48                       | [0.26-0.75]     | 0.26                   | [0.16-0.38]     | P=0.034 | 0.36                       | [0.23-0.61]     | 0.26                   | [0.17-0.45]      | NS      |
| IL-23                                                                    | 0                          | 0               | 0                      | 0               | NS      | 0                          | 0               | 0                      | 0                | NS      |
| IL-27                                                                    | 353.29                     | [240.73-480.73] | 343.32                 | [209.17-375.62] | NS      | 373.68                     | [281.87-480.49] | 293.72                 | [206.67-351.10]  | NS      |
| IL-31                                                                    | 0.01                       | [0-0.03]        | 0.01                   | [0 -0.05]       | NS      | 0.01                       | [0.00-0.03]     | 0.01                   | [0.00-0.03]      | NS      |
| MIP-3α                                                                   | 1.31                       | [0.78-2.63]     | 0.94                   | [0.58-1.21]     | NS      | 1.31                       | [0.70-2.65]     | 1.09                   | [0.65-1.22]      | NS      |

**Table 3.2 Baseline concentrations, median [IQR] of 54 inflammatory markers categorised by patient status i.e., persistent disease activity or steroid free remission at week 14 and at week 30.** p values comparing patients with persistent disease with steroid free remission at week 14 and week 30 were calculated using the Mann Whitney U for continuous variables and Chi squared categorical variables

In univariate analyses, the baseline concentrations of eight inflammatory markers were associated with steroid free remission. Higher baseline levels of IL-7, IL-22 and VEGF-C were associated with week 14 persistent disease activity. Higher baseline levels of IL-10 with week 30 persistent disease activity. Conversely, higher baseline levels of TNF- $\beta$  were associated with week 14 SFR and higher baseline levels of MCP-4, MDC and IL-4 with week 30 SFR. **(Figure 3.3)**

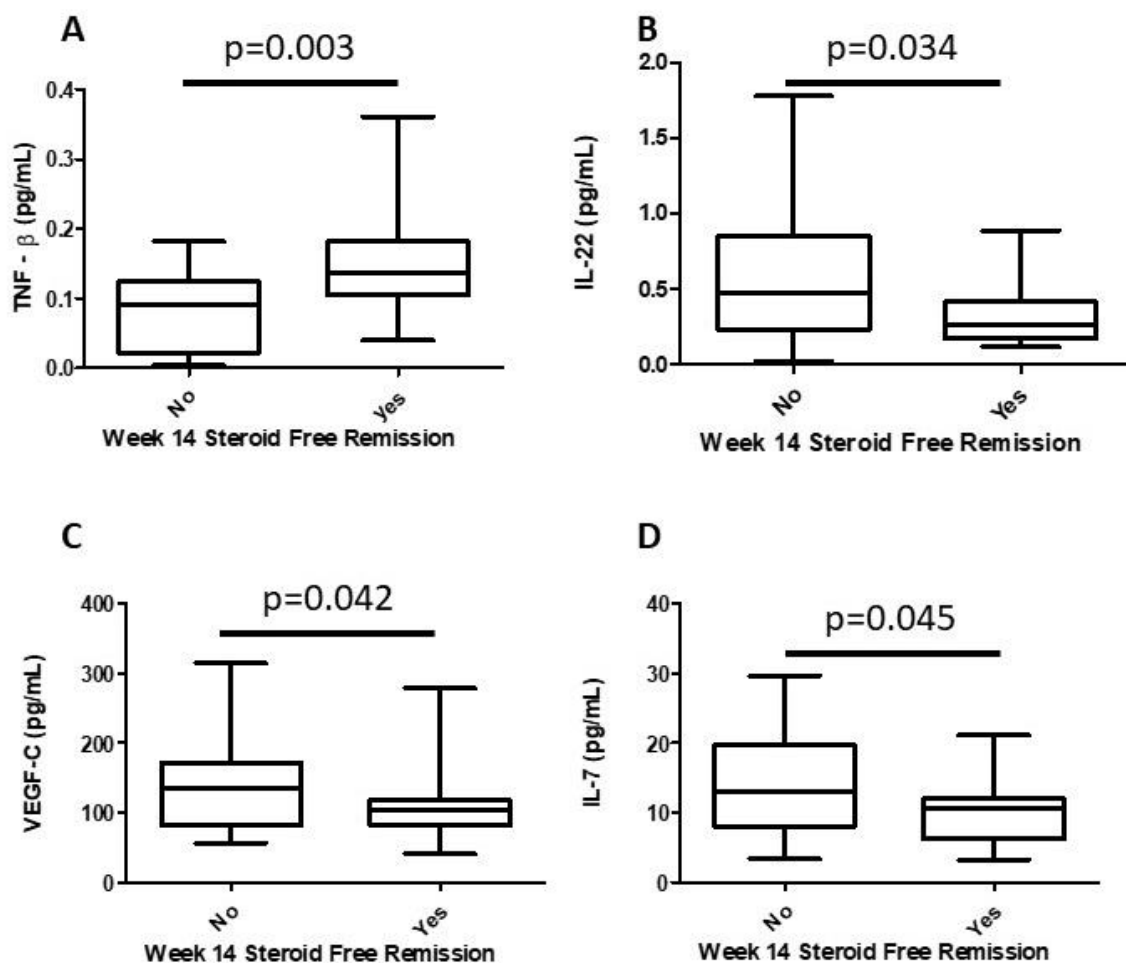


**Figure 3.2. Boxplots showing the Median, IQR and range of the baseline concentrations of TNF- $\beta$ , IL-22, VEGF-C and IL-7 in those with and without week 14 steroid free remission.**

In univariate analysis TNF- $\beta$ , was significantly higher (p=0.003) and those IL-22, VEGF-C and IL-7 were significantly lower (p=0.034, p=0.042, p=0.045, respectively) in those with steroid free remission at week 14 compared to those with persistent disease activity. p values were calculated using the Mann Whitney U test.

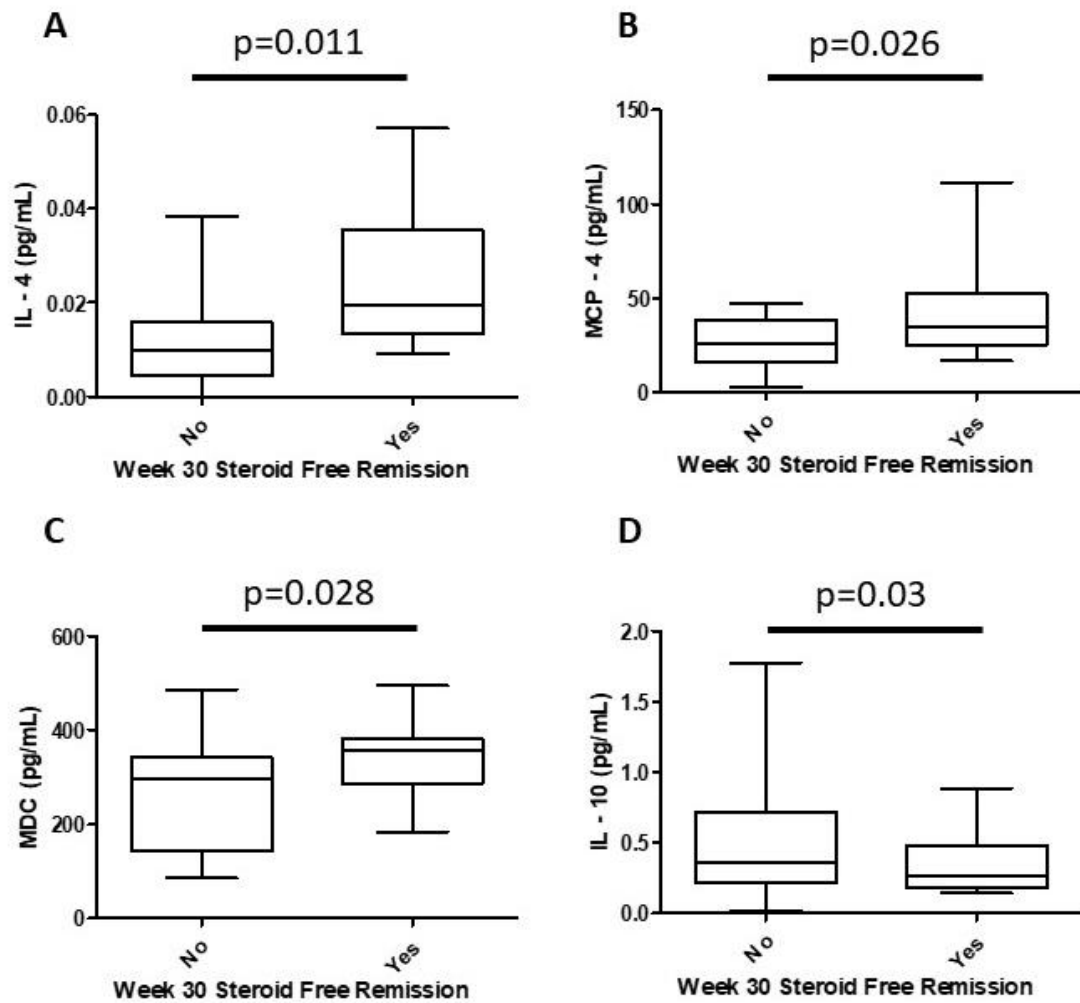


**Figure 3.3.** Boxplots showing the median, IQR and range of the baseline concentration of IL-4, MDC, IL-10 and MCP-4 in those with and without week 30 steroid-free remission. In univariate analysis the baseline concentrations of IL-4, MDC, and MCP-4, were significantly higher ( $p=0.011$ ,  $p=0.026$ , and  $p=0.028$  respectively) and those IL-10 significantly lower ( $p=0.03$ ) in those with steroid free remission at week 30 compared to those with persistent disease activity.  $p$  values were calculated using the Mann Whitney U test.

#### 3.4.4 Baseline Concentrations of Serum Inflammatory Markers and Vedolizumab

##### Treatment Persistence

Continuation of VDZ therapy is considered an objective hard endpoint that is independent of clinical symptoms. Although no single inflammatory marker was clearly identified as predictive of SFR, recognising the stringency of the Benjamini–Hochberg procedure for multiple test correction and the inherent potential to miss a relevant association, we examined whether any of the potential biomarkers identified in the univariate analysis were predictive of VDZ therapy persistence or discontinuation.

During the study, 23 (58%) patients discontinued VDZ. The median [range] time to VDZ discontinuation was 9.3 [0.5–38.7] months. The eight inflammatory markers nominally associated with either week 14 or week 30 SFR were assessed for association with VDZ treatment persistence or discontinuation. Kaplan-Meier survival curves were used to estimate the likelihood of remaining on VDZ according to the median baseline concentration of each analyte. Shown in **Figure 3.4** are the cumulative survival curves for the 19 patients with the highest concentrations compared with the those of 19 patients with the lowest concentrations of the inflammatory markers. No associations with VDZ treatment persistence were identified.

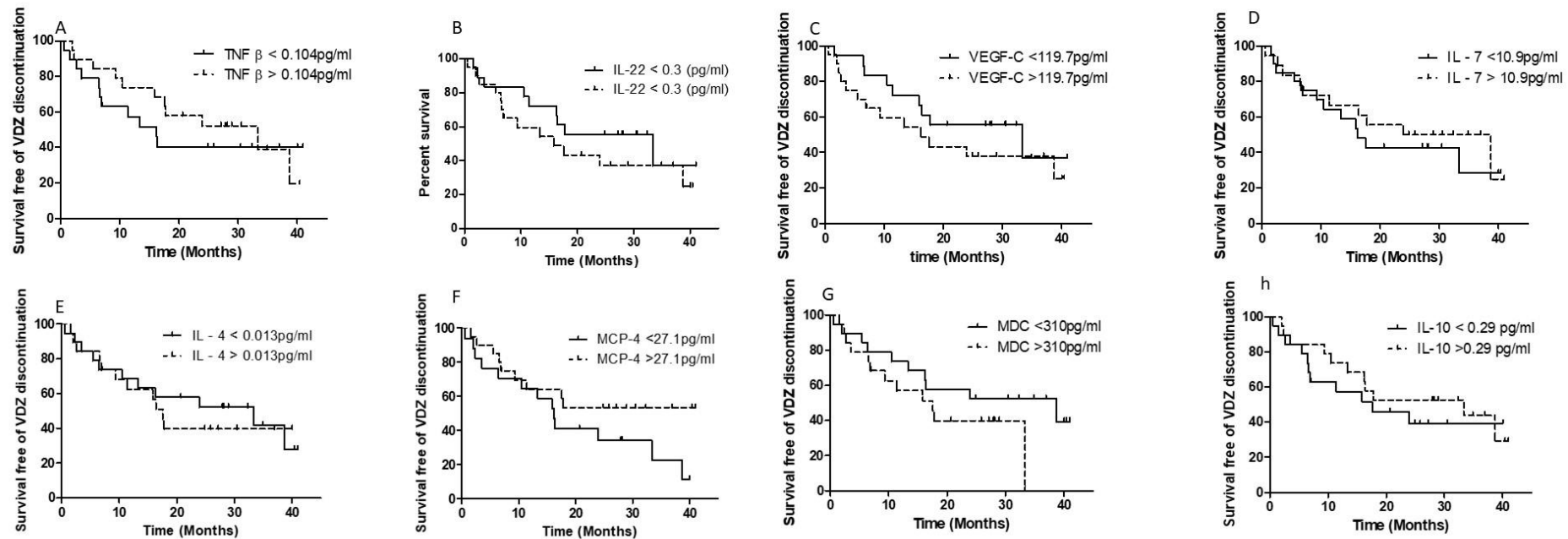


**Figure 3.4: Kaplan-Meier Survival Curve Analyses showing persistence on vedolizumab therapy (in months) by inflammatory marker concentration.** Survival curves for 19 patients with inflammatory marker concentrations above the median are compared with those for the 19 patients with concentrations below the median for each of the eight inflammatory markers identified in univariate analysis as associated with either week 14 or week 30 steroid free remission. The medians were as follows; **A**) TNF- $\beta$ , 0.104pg/mL; **B**) IL-22, 0.3pg/mL; **C**) VEGF, 119.7pg/mL; **D**) IL-7, 10.9pg/mL; **E**) IL-4, 0.013pg/mL; **F**) MCP-4, 27.1pg/mL **G**) MDC, 310pg/mL; and **H**) IL-10, 0.29pg/mL No significant differences were detected in persistence on therapy related to any of the tested inflammatory markers. Significance was tested using the Log Rank Test.

### 3.4.5 Baseline Concentrations of Serum Inflammatory Markers and their Association with Therapeutic Vedolizumab Concentration.

Based on a recent meta-analysis, a VDZ trough level of  $>12\mu\text{g/mL}$  during maintenance therapy is considered adequate and may be predictive of better clinical response.(341) As reported in **Chapter 2**, a low serum VDZ trough level is associated with treatment failure. Similar findings i.e., that trough levels are associated with treatment outcomes, have been reported related to other biologic therapies e.g., with infliximab and adalimumab.(232, 380) The eight inflammatory markers nominally identified in univariate analysis as associated with SFR at either 14 or 30 week were further assessed to see if they were predictive of adequacy of serum VDZ trough levels.

Maintenance trough VDZ levels obtained prior to the fifth infusion were available on 34 patients, 18 of whom had levels  $>12\mu\text{g/mL}$ . Of the eight potential biomarkers, only lower baseline IL-7 concentrations were associated with adequate maintenance VDZ trough levels ( $p=0.014$ ) (**Figure 3.5**).

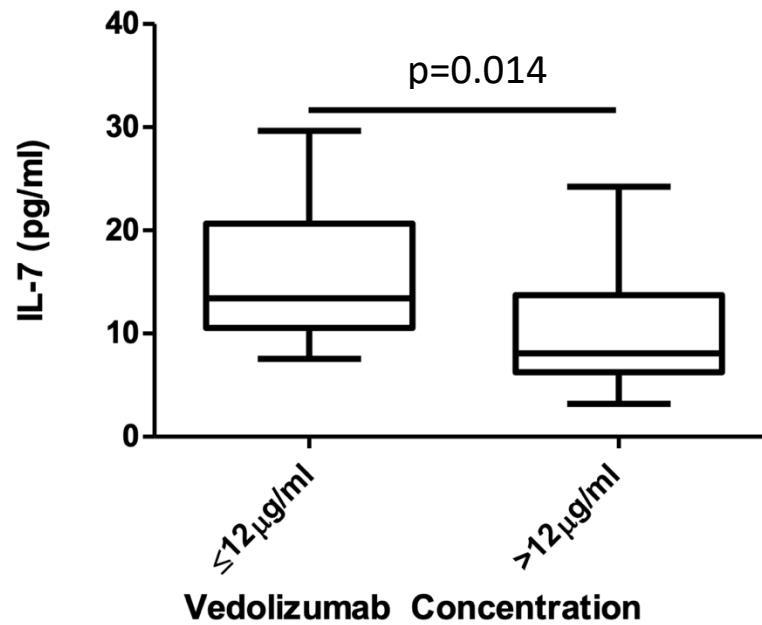


**Figure 3.5** The median, IQR and Range of Baseline IL-7 Concentrations (pg/mL) are Shown for those with VDZ ≤12µg/mL and >12µg/mL following Induction.

IL-7 levels were significantly lower in those achieving concentrations >12µg/mL. p values were calculated using the Mann Whitney U test for continuous variables

#### 3.4.6 Concentrations of Serum Inflammatory Markers Immediately Prior to, and at week 6 of Vedolizumab Therapy.

The concentrations of 54 inflammatory markers were measured in patients prior to and following VDZ induction and the change from baseline assessed. In univariate analysis the change in the concentrations of seven differed significantly from baseline. Eotaxin ( $p=0.001$ ), eotaxin-3 ( $p=0.001$ ), TNF- $\beta$  ( $p=0.007$ ), TARC ( $p=0.015$ ), BFGF ( $p=0.034$ ), and MIP-1 $\beta$  ( $p=0.043$ ) all increased whereas IL-15 concentration decreased ( $p<0.001$ ) (**Table 3.6**)

| Concentrations of Serum Inflammatory Markers (pg/mL), Median [IQR] |                       |                  |         |                  |          |
|--------------------------------------------------------------------|-----------------------|------------------|---------|------------------|----------|
|                                                                    | Pre-Induction, Week 0 |                  | Week 6  |                  | P Value  |
| Angiogenesis Panel                                                 |                       |                  |         |                  |          |
| bFGF                                                               | 6.15                  | [0.17-27.70]     | 9.41    | [0.52-62.87]     | P=0.034  |
| Flt-1                                                              | 95.99                 | [40.67-273.10]   | 89.81   | [28.24-216.02]   | NS       |
| PlGF                                                               | 3.83                  | [2.53-9.18]      | 4.07    | [2.54-8.31]      | NS       |
| Tie-2                                                              | 1829.68               | [954.37-2577.28] | 1879.40 | [953.38-2473.25] | NS       |
| VEGF                                                               | 244.22                | [42.06-842.85]   | 266.14  | [67.15-1180.51]  | NS       |
| VEGF-C                                                             | 115.00                | [0.00-700.74]    | 112.29  | [11.22-466.17]   | NS       |
| VEGF-D                                                             | 927.20                | [316.87-1894.65] | 925.93  | [238.04-1883.65] | NS       |
| Chemokine Panel 1                                                  |                       |                  |         |                  |          |
| Eotaxin                                                            | 80.82                 | [17.86-341.44]   | 116.43  | [37.16-424.77]   | P=0.0012 |
| Eotaxin-3                                                          | 1.41                  | [0.03-10.02]     | 2.39    | [0.27-12.01]     | P=0.0013 |
| IL-8                                                               | 29.42                 | [0.00-158.65]    | 24.76   | [0.00-4583.46]   | NS       |
| IP-10                                                              | 61.33                 | [14.24-873.72]   | 61.58   | [11.63-444.68]   | NS       |
| MCP-1                                                              | 66.33                 | [17.87-269.03]   | 74.42   | [34.31-240.73]   | NS       |
| MCP-4                                                              | 28.42                 | [3.03-111.57]    | 35.49   | [8.70-128.23]    | NS       |
| MDC                                                                | 317.93                | [85.11-495.03]   | 276.32  | [90.13-728.34]   | NS       |
| MIP-1α                                                             | 4.57                  | [1.48-21.44]     | 4.88    | [2.53-304.68]    | NS       |
| MIP-1β                                                             | 32.36                 | [8.00-262.24]    | 34.00   | [9.82-388.59]    | P=0.043  |
| TARC                                                               | 58.30                 | [3.74-267.64]    | 79.90   | [7.94-322.36]    | P=0.015  |
| Chemokine Panel 2                                                  |                       |                  |         |                  |          |
| IL-17A/F                                                           | 0.49                  | [0.00-2.38]      | 0.73    | [0.00-2.87]      | NS       |
| IL-17B                                                             | 0                     | [0.00-10.04]     | 0.01    | [0.00-5.44]      | NS       |
| IL-17C                                                             | 0                     | [0.00-1.99]      | 0       | [0.00-3.71]      | NS       |
| IL-17D                                                             | 1.99                  | [0.00-8.12]      | 2.28    | [0.00-12.55]     | NS       |
| IL-1RA                                                             | 100.88                | [39.97-540.18]   | 112.08  | [42.44-490.14]   | NS       |
| IL-3                                                               | 0                     | [0.00-4.64]      | 0       | [0.00-3.42]      | NS       |
| IL-9                                                               | 0                     | [0.00-0.13]      | 0       | [0.00-0.40]      | NS       |
| TSLP                                                               | 0.25                  | [0.00-0.97]      | 0.31    | [0.00-0.95]      | NS       |
| Cytokine Panel 1                                                   |                       |                  |         |                  |          |
| GM-CSF                                                             | 0                     | [0.00-0.11]      | 0       | [0.00-0.69]      | NS       |
| IL-12/IL-23p40                                                     | 72.19                 | [14.90-566.25]   | 84.17   | [15.01-926.78]   | NS       |
| IL-15                                                              | 1.41                  | [0.70-5.49]      | 1.23    | [0.73-5.16]      | P=0.0004 |
| IL-16                                                              | 98.02                 | [45.42-253.15]   | 97.29   | [38.72-244.23]   | NS       |
| IL-17A                                                             | 3.13                  | [0.89-9.25]      | 2.80    | [0.52-12.04]     | NS       |
| IL-1α                                                              | 0                     | [0.00-2.99]      | 0       | [0.00-2.57]      | NS       |
| IL-5                                                               | 0                     | [0.00-1.56]      | 0       | [0.00-3.83]      | NS       |
| IL-7                                                               | 10.58                 | [3.18-29.66]     | 10.93   | [3.69-26.59]     | NS       |
| TNF-β                                                              | 0.10                  | [0.00-0.36]      | 0.13    | [0.01-0.34]      | P=0.0077 |
| Proinflammatory Cytokine Panel                                     |                       |                  |         |                  |          |
| IFN-γ                                                              | 3.02                  | [1.59-6.22]      | 3.25    | [1.77-6.1]       | NS       |
| IL-10                                                              | 0.32                  | [0.22-0.54]      | 0.29    | [0.19-0.53]      | NS       |
| IL-12p70                                                           | 0.12                  | [0.06-0.22]      | 0.09    | [0.06-0.23]      | NS       |
| IL-13                                                              | 0.36                  | [0.22-0.49]      | 0.34    | [0.24-0.47]      | NS       |
| IL-1β                                                              | 0.02                  | [0.00-0.09]      | 0.02    | [0.00-0.07]      | NS       |
| IL-2                                                               | 0.08                  | [0.05-0.16]      | 0.08    | [0.06-0.11]      | NS       |
| IL-4                                                               | 0.01                  | [0.01-0.02]      | 0.02    | [0.01-0.03]      | NS       |
| IL-6                                                               | 0.74                  | [0.48-1.39]      | 0.77    | [0.44-1.42]      | NS       |
| IL-8                                                               | 12.32                 | [8.83-21.72]     | 12.14   | [7.49-17.77]     | NS       |
| TNF-α                                                              | 1.82                  | [1.37-2.53]      | 1.93    | [1.4-2.59]       | NS       |

| Serum Cytokine Concentrations (pg/mL), Median [IQR] |                       |                    |         |                    |         |
|-----------------------------------------------------|-----------------------|--------------------|---------|--------------------|---------|
|                                                     | Pre-Induction, Week 0 |                    | Week 6  |                    | P Value |
| TH 17 Panel                                         |                       |                    |         |                    |         |
| IL-17A GenB                                         | 0.25                  | [0.00-2.16]        | 0.19    | [0.00-3.11]        | NS      |
| IL-21                                               | 0                     | [0.00-1.07]        | 0       | [0.00-0.81]        | NS      |
| IL-22                                               | 0.26                  | [0.01-1.78]        | 0.31    | [0.00-1.03]        | NS      |
| IL-23                                               | 0                     | [0.00-0.00]        | 0       | [0.00-0.25]        | NS      |
| IL-27                                               | 344.53                | [86.18-697.55]     | 327.48  | [115.15-701.31]    | NS      |
| IL-31                                               | 0                     | [0.00-0.20]        | 0.00    | [0.00-0.16]        | NS      |
| MIP-3α                                              | 1.14                  | [0.00-9.78]        | 1.22    | [0.06-12.88]       | NS      |
| Vascular Injury Panel 1                             |                       |                    |         |                    |         |
| CRP                                                 | 4245.25               | [361.42-145889.61] | 4953.39 | [238.39-63135.15]  | NS      |
| ICAM-1                                              | 629.76                | [287.25-1191.08]   | 617.54  | [298.22-962.72]    | NS      |
| SAA                                                 | 8692.64               | [551.95-204518.08] | 5179.22 | [578.80-166653.35] | NS      |
| VCAM-1                                              | 583.40                | [335.19-1277.38]   | 577.27  | [320.48-1194.99]   | NS      |

**Table 3.3 Concentrations (pg/mL) of 54 inflammatory markers (median [IQR]) at baseline and at week 6 of vedolizumab induction.**

In univariate analysis significant increases were seen in six; bFGF, eotaxin, eotaxin-3, MIP-1 $\beta$ , TARC and TNF- $\beta$  ( $p=0.034$ ,  $0.0012$ ,  $0.0013$ ,  $0.043$ ,  $0.015$  and  $0.0077$  respectively) and a significant decrease noted in one; IL-15 ( $p=0.0004$ ) in association with VDZ therapy. Significance was assessed using the Wilcoxon sign-rank test for paired continuous data.

Following multiple test correction the changes in three inflammatory markers; IL-15, eotaxin, and eotaxin-3 remained significant. IL-15 decreased significantly from baseline in association with VDZ induction therapy ( $p=0.0004$ ) whereas serum concentration of eotaxin, and eotaxin-3 concentrations increased ( $p=0.0012$  and  $0.0013$  respectively) (**Figure 3.6**)

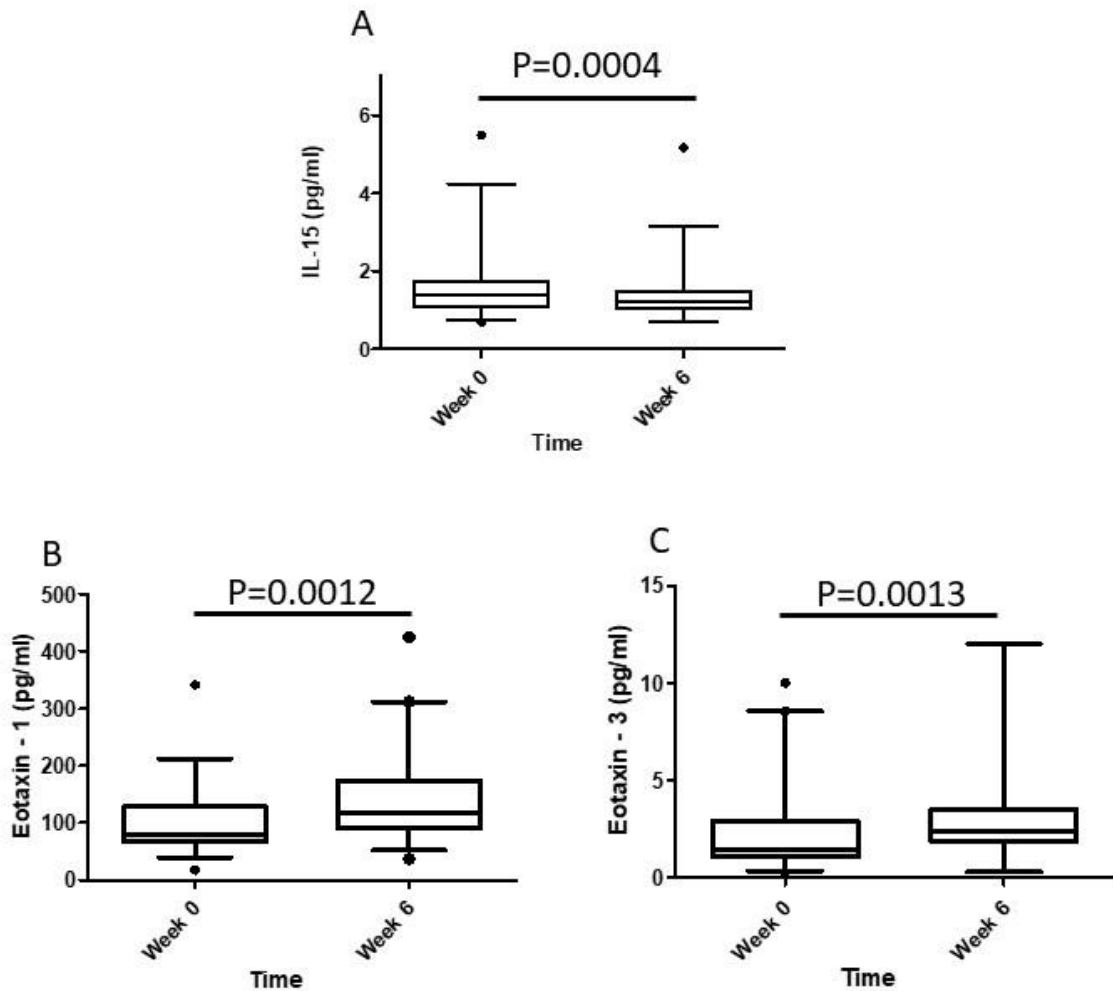


**Figure 3.6. Boxplots showing the median, IQR and range of Eotaxin, Eotaxin-3 and IL-15 (pg/mL) at baseline (week 0) and at week 6. Concentrations of IL-15 decreased ( $p=0.0004$ ) and those of eotaxin and eotaxin-3 increased ( $p=0.0012$  and  $p=0.0013$  respectively). p values were calculated using the Wilcoxon sign-rank test for paired continuous data. s**

### 3.5 Discussion

Vedolizumab, while an important addition to the treatment options for IBD patients, is nonetheless associated with a significant treatment failure rate. Gaining a better understanding of its mechanisms of action at the local tissue level and identifying circulating serum biomarkers indicative of likely treatment success is an attractive prospect as it would enable more effective patient selection for VDZ treatment.

For other inflammatory diseases, a number of different cytokines or cytokine profiles have been identified as biomarkers of disease activity, progression and therapeutic response.(381-384) A number of inflammatory proteins; cytokines, chemokines and growth factors, act as important mediators of cell to cell communication and control of the inflammatory process. No clear biomarker of IBD treatment response to VDZ has yet been identified. Thus, research relating to the role of inflammatory proteins in IBD and how therapeutic agents such as VDZ might affect them is warranted.

To add to the current knowledge base, we prospectively determined serum concentrations of 54 inflammatory markers that included a broad array of cytokines, chemokines and growth factors, in patients undergoing VDZ induction. We examined the correlation of baseline (pre-treatment) levels with therapeutic response and the effect of VDZ induction on the serum concentration of each inflammatory marker tested.

#### *Inflammatory proteins as potential biomarkers of IBD disease response*

In the primary analysis, the baseline concentrations of eight out of fifty four inflammatory markers were found to be significantly associated with clinical response. While the statistical threshold for significance was not achieved when adjusted for multiplicity, that some or all of these inflammatory markers might be clinically relevant is biologically plausible as many of the eight identified are known to be associated with leukocyte migration.

TNF- $\beta$  is one cytokine that may be of interest as a biomarker of likely VDZ response. Those with SFR at 14 weeks had higher baseline levels of TNF- $\beta$  ( $p=0.003$ ). At 30 weeks, baseline TNF- $\beta$

concentrations did not differ significantly between those with and without SFR, however a trend toward higher levels was noted and TNF- $\beta$  concentrations were increased following VDZ induction ( $p=0.0077$ ).

TNF- $\beta$ , also known as lymphotoxin- $\alpha$ , has many functions in lymphocyte development. It shares significant homology (30%) with TNF- $\alpha$ (385) and like TNF- $\alpha$  binds to TNF Receptor I and TNF Receptor II.(386) It appears to play a critical role in the development of lymphoid organs(387) and can activate inflammatory markers via the NF $\kappa$ B signalling pathway.(388) In two independent murine models of colitis, soluble lymphotoxin- $\beta$  receptor, a competitive inhibitor of membrane bound TNF- $\beta$ , was found to attenuate colitis development.(389) However, to our knowledge no study has linked baseline TNF- $\beta$  with disease progression in IBD. High levels of baseline TNF- $\beta$  may indicate a disease phenotype that is dependent on the MAdCAM-1  $\alpha_4\beta_7$  pathway and could potentially be used to identify a phenotype that is more susceptible to VDZ inhibition of that pathway.

IL-4, an inflammatory cytokine is known to drive a Th2 response and is inhibitory of Th1 cell activity. In this study, baseline IL-4 levels did not discriminate between those with and without SFR at 14 weeks however, those with SFR at week 30 had higher baseline levels ( $p=0.011$ ) compared to those with persistent disease. As IL-4 activated macrophages are associated with improved wound healing and reduced inflammation in colitis mouse model (390), further study of this cytokine in IBD is warranted.

Conversely, significantly higher baseline concentrations of IL-22 were found in those who had persistent disease at 14 weeks ( $p=0.034$ ) and were non-significantly higher in those with persistent disease at 30 weeks. VDZ induction did not affect the IL-22 concentrations. IL-22, a member of the IL-10 cytokine family,(391) is expressed by CD4 memory T cells, TH1 cells, and NK cells.(392) Expression of the IL-22 receptor (IL-22R) is found in epithelial cells, keratocytes, and hepatocytes, facilitating the interaction of IL-22 with the innate immune system.(393) Expression of IL-22 differs between the cells in the small and large intestine. It is thought that It may have a role in protecting

the epithelial barrier against enteric microorganisms.(394) Rarely expressed in the healthy colon, it is present in the inflamed colon.(395) In IBD it has been shown to have variable expression. In mouse models and in human disease, IL-22 expression is higher in CD than in UC. In this study, in contrast to TNF- $\beta$ , higher baseline IL-22 levels found in those with persistent disease. This and the lack of effect of VDZ induction on IL-22 levels may reflect greater inflammation and identify a more severe disease phenotype that is more resistant to VDZ.

Similar to IL-22, the baseline levels of VEGF-C and IL-7 were found to be significantly higher in those with persistent disease at 14 weeks and were higher, but did not reach the threshold of significance, for those with persistent disease at 30 weeks. VDZ induction also failed to affect their secretion levels. VEGF-C is a growth factor that is involved in lymphatic drainage. In colitis mouse models, over expression of VEGF-C increases intestinal inflammation.(396)

IL-7 is a potent immunostimulatory cytokine and has been recognised as an important pro-inflammatory mediator of chronic autoimmune disease.(397) Overexpression of IL-7 was associated with the development of colitis in a transgenic mouse model.(398) Lee et al., using gene profiling of CD8+ T cells, identified that over expression the IL-7 signalling pathway could be an important element contributing to treatment-refractory, relapsing, or chronically active disease.(399) It is therefore plausible that the higher baseline serum levels may correlate with persistent disease activity and indicate a more refractory diseases phenotype. It is of note that in our study lower baseline IL-7 levels were significantly associated with therapeutic trough drug levels during maintenance, an accepted predictor of better disease outcome.

IL-7 and its receptor have been considered as potential targets for treatment of IBD however clinical trials have yielded disappointing results.(400) Decreased IL-7 expression in the tissue microenvironment of UC biopsies has been reported to be associated with UC disease progression.(401) Gaining an understanding of the distinctive roles of serum and tissue levels of IL-7 will be important. Tomita et al in addressing this paradox concluded that systemic IL-7 but not intestinal IL-7 is essential for the development and perpetuation of colitis.(402)

Our findings suggest that IL-22, VEGF-C, and IL-7 are potential biomarkers of a more severe disease that may not be as responsive to VDZ therapy. It is also noteworthy, that over time in contrast to TNF- $\beta$  fewer patients with higher baseline IL-22 and VEGF-C remained on treatment. The Kaplan Meier analysis failed to detect any significant difference in VDZ persistence according to whether the baseline cytokine level fell above or below the median levels for all patients.

Our data also challenges the existing perception regarding the action of some inflammatory markers in the microenvironment. IL-10, a regulatory cytokine that inhibits pro inflammatory cytokine release including TNF- $\alpha$ , IL-4, and eotaxin,(403) has been investigated as therapy for colitis in mouse models. Randomised controlled trials of recombinant IL-10 for moderately active CD in humans initially showed an increase response in patient treated with IL-10 when compared with placebo however it was not significantly more effective than placebo for induction of remission.(404) Against this background, higher baseline levels of IL-10 might be anticipated to predict SFR, however such was not found. Among our patients, baseline IL-10 levels were higher in those with persistent disease with a significant difference noted between those with and without SFR at 30 weeks( $p=0.03$ ). VDZ did not affect IL-10 secretion.

Macrophage derived chemoattractant factor (MDC) and Human monocyte chemoattractant protein (MCP-4), are two chemokines that are potent chemoattractants for immature dendritic cells and lymphocytes.(405, 406) The baseline levels of each were lower ( $p=0.026$  and  $p=0.028$  respectively) in those with persisting disease at week 30 supporting the hypothesis that at least in some patients pathways other than lymphocyte migration are involved in the inflammatory process in IBD. Further support to this is given by the fact that VDZ induction, where the main mechanism of action is thought to related to inhibition of lymphocyte migration, had no impact on the levels of MDC and MCP-4. To our knowledge, no consistent biomarker of IBD disease progression or therapeutic response has been clearly identified.

In a cohort of 22 VDZ treated patients with refractory CD, Holmer et al. evaluated a range of potential biomarkers for associations with endoscopic remission. They used the same vascular

injury panel, as in this study, (ICAM-1, VCAM-1, SAA-1, and CRP) in addition to the evaluation of MAdCAM-1, TNF- $\alpha$  and soluble-  $\alpha_4\beta_7$  (s-  $\alpha_4\beta_7$ ). The study had both clinical endoscopic remission as the endpoints. Based on their findings of higher concentrations of VCAM-1 and ICAM-1 early in treatment, that these associated with subsequent endoscopic remission, and that these biomarkers increased more from baseline to maintenance in remitters compared with non-remitters, they hypothesised that serum concentrations of VCAM-1 or ICAM-1 during induction might be useful predictors of response to therapy. In our study neither baseline levels of ICAM-1 nor VCAM-1 were predictive of clinical status at 14 or at 30 weeks nor were the concentrations of either altered by VDZ induction therapy. Whereas Holmer et al. reported that concentrations of MAdCAM-1 decreased and serum s- $\alpha_4\beta_7$  increased following VDZ infusion. In that study, consistent with the findings reported in our work, neither TNF- $\alpha$  nor any of the vascular injury cytokines were affected by VDZ induction out to week 26.(384)

A separate study from the same investigator group focused on the same range of potential biomarkers in 32 patients with UC and similar findings were reported. No baseline biomarker was associated with either endoscopic or clinical remission. VDZ induction led to decreased MAdCAM-1 and increased soluble  $\alpha_4\beta_7$  integrin and maintenance concentrations of soluble  $\alpha_4\beta_7$  integrin were higher in disease remitters than those with persistent activity. Changes in other cytokines were inconsistent with lower TNF- $\alpha$  concentrations at week 26 but not week 6 whereas ICAM-1 concentrations were significantly lower at week 6 but not at week 26; inconsistencies which, as pointed out by the authors, might have been contributed to by small sample size.(383)

Another study reported findings from 28 IBD patients who had previously failed anti-TNF therapy and who were due to start VDZ therapy. Twenty of 28 (71%) failed to achieve a clinical response to VDZ by week 20. The study included 37 different inflammatory markers, including IL-10, IL-2 and IL-22 but not IL-7, TNF- $\beta$ , or VEGF-C. Using univariate analysis, without correction for multiplicity, the authors reported that circulating IL-6 was higher among non-responders and osteocalcin higher among responders to VDZ.(382)

A study of 32 UC patients had nine serum biomarkers assessed during VDZ induction therapy, found that higher baseline levels of IL-6 and IL-8, followed by their significant decline over the ensuing 6 weeks was significantly associated with mucosal healing at week 54. The authors concluded that serum patterns of IL-6 and IL-8 at baseline and their pattern over the following weeks could be useful in predicting disease outcome.(407) In our mixed patient cohort of CD and UC patients, we were unable to confirm any association of either IL-6 or IL-8 with disease status at 14 or 30 weeks, nor were any significant changes in the concentration of either cytokine documented at 6 weeks following induction therapy.

#### *Cytokines and response to VDZ induction*

In our study, significant changes were identified in seven markers of inflammation following six weeks of VDZ therapy. Of these seven: TNF- $\beta$ , bFGF, eotaxin, eotaxin-3, MIP-1 $\beta$ , TARC and IL-15, only baseline levels of TNF- $\beta$  correlated with disease status at 14 weeks. After correction for multiplicity only changes in IL-15, eotaxin and eotaxin-3 remained statistically significant.

IL-15, is an autocrine regulator of proinflammatory chemokine production in macrophages and promotes cytoskeletal rearrangement that is important for movement and extravasation in neutrophils. It induces T cell proliferation, chemotaxis and protects cells from apoptosis. It is considered a marker of the Th1 type inflammatory response. VDZ, in inhibiting chemotaxis, may contribute to decreased IL-15 production.

IL-15 is highly expressed in the inflamed mucosa of patients undergoing a flare of CD and UC.(408-410) Transgenic mice that over express IL-15 in enterocytes develop severe duodenal and jejunal inflammation.(411) Inhibition of IL-15 in explant studies results in decreased cytokine production.(412) In this study serum levels of IL-15 decreased in response to VDZ induction.

A study of IL-15 mRNA in patients during infliximab induction showed no effect of infliximab on IL-15 mRNA concentrations, either in the serum or the tissue micro-environment.(413) This may indicate that the changes in concentration of IL-15 are VDZ specific.

The importance of IL-15 as a potential therapeutic target was shown in mouse model studies of eosinophilic oesophagitis and refractory coeliac disease.(414) IL-15 knock out mice have also been shown to be less susceptible to dextran sodium sulphate acute and chronic colitis than wild-type animals.(415) While in this study we were unable to demonstrate any correlation between baseline IL-15 level and disease status at 14 or at 30 weeks, the reduction in IL-15 in response to VDZ induction is significant and is possibly highlighting a mechanism of action of VDZ beyond inhibition of lymphocyte migration. A pattern of decline in IL-15 during induction may prove a useful biomarker in detecting early response to VDZ. Further investigations into IL-15 and its role in IBD could lead to development of a novel therapeutic target for IBD.

Eotaxins, another group of chemokines that selectively attract eosinophils, are constitutently expressed in the gastrointestinal tract.(416, 417). Over-expression of eotaxin has been shown to induce intestinal eosinophilia.(418) Increased serum eotaxin has been found in UC and in CD(419, 420) and to be present in tissues of patients with active UC and CD.(421) Targeting eotaxin with neutralising monoclonal antibodies in a mouse model colitis can ameliorate the severity of disease activity and improve histological inflammation.(421, 422) In a transgenic mouse study of eotaxin deficient mice, the reinsertion of the eotaxin gene in mice reversed paucity of intestinal eosinophils, however this process is dependent on the presence of the  $\alpha_4\beta_7$  integrin.(418) Our study shows an increase of both eotaxin and eotaxin-3 in IBD patients during VDZ induction. By removing one method of eosinophil recruitment i.e., blocking  $\alpha_4\beta_7$ , compensatory over-expression of eotaxin may occur.

It is notable that neither eotaxin nor IL-15 appear to directly correlate with the degree of inflammation. If the changes in either chemokine were simply reflective of disease activity rather than directly related to VDZ, one would expect their levels to associate with either week 14 or week 30 steroid free remission.

### 3.6 Study Strengths and Limitations

Study strengths included the well characterised patient cohort, the completeness of the data at each of the evaluation, the number of inflammatory secreted proteins analysed, and that both patients with UC and CD were included. A greater array of inflammatory markers were included for evaluation compared to other similar studies.(382-384) Statistical analysis yielded some interesting potential associations, however when stringent multi-test correction was applied, few retained statistical significance. Although participant numbers were very good for this type of study, much larger numbers would be needed to validate and confirm associations postulated based on this data. The participant numbers may not have been sufficient to detect small, yet clinically meaningful differences in biomarkers. In our study the inflammatory markers were assayed at just two time points, baseline and prior to the week 6 infusion. It would be of interest to narrow the focus, concentrating on those markers identified as of interest and to track their changes over a number of different time points throughout the treatment course and to examine for correlation with treatment outcome.

### 3.7 Conclusion

In this study of 39 patients undergoing VDZ therapy we were unable to confirm any single biomarker as predictive of SFR, however biologically plausible associations with a number of such inflammatory markers were identified. Candidates for further research include TNF- $\beta$ , IL-22, VEGF-C, IL-7, IL-15, eotaxin and eotaxin-3.

Baseline higher TNF- $\beta$  may be predictive of better disease outcome and continuation of VDZ therapy whereas for other cytokines specifically IL-22, VEGF-C and IL-7, higher baseline levels associated with disease persistence at 14 weeks although this was not confirmed with respect to week 30. Supporting this concept is the finding that lower baseline serum IL-7 levels associated with therapeutic VDZ levels during maintenance, a known predictor of better treatment outcome. A significant effect of VDZ on inflammatory markers was noted only for three; IL-15, eotaxin and eotaxin-3, and raised the hypothesis that a pattern of decline of IL-15 early in induction might be

predictive of treatment success. While the effect of VDZ on eotaxin and eotaxin 3 levels might have been unanticipated, these findings raise further questions as to the role of those chemokines in IBD. Further study of larger numbers of patients focusing on the inflammatory markers identified in this study as of interest is warranted. The work here adds to the body of knowledge relating to VDZ and its effect on a wider array of inflammatory markers than have been previously tested. Results suggest that a pattern of lower baseline concentrations of TNF- $\beta$  and IL-7 and early decline in IL-15 might be predictors of a better therapeutic response to VDZ whereas higher baseline concentrations of IL-22, VEGF-C and IL-7 might identify those more likely to have persistent disease. Further research will be required to confirm these findings to determine clinical utility.

## **Chapter 4.0:**

Patient Derived Tissue Explants: A  
Potentially Useful Tool in the  
Investigation of Vedolizumab and its'  
Impact on the Gut Tissue  
Microenvironment.

## 4.1 Introduction

Following the development of anti-TNF therapy, biologic agents have rapidly become the mainstay treatment of moderate to severe Ulcerative Colitis (UC). While biologic therapies are effective, more than half of patients fail to achieve complete remission and up to 70% of patients will require change of therapy within 5 years.(2-5) Newer biologic agents include ustekinumab that targets IL-12/IL-23 and vedolizumab (VDZ), a humanised monoclonal antibody that specifically recognises and inhibits the  $\alpha_4\beta_7$  integrin. Even with the newer agents up to one third of patients may experience primary treatment failure and in approximately one third of responders the initial response is not sustained.

Vedolizumab was developed to inhibit lymphocyte migration to the gut through binding the  $\alpha_4\beta_7$  integrin present on lymphocytes primed to migrate to the gut.(7) Higher trough concentrations of VDZ during induction are associated with persistence of treatment and lower week 6 levels are associated with treatment failure (see **Chapter 2**). Yet, VDZ attains similar levels of  $\alpha_4\beta_7$  saturation even at very low VDZ concentrations and  $\alpha_4\beta_7$  saturation levels do not differ between those with or without mucosal healing.(324) *In vitro* analysis shows that a concentration of 1 $\mu$ g/mL VDZ is high enough to achieve saturation of T cells, eosinophils, and circulating macrophages.(324, 348) Some studies have questioned the effectiveness of VDZ in inhibiting lymphocyte migration into the intestine as VDZ does not affect the intestinal repertoire of T cells in colonic biopsies of UC and CD patients.(378) Migration of CD8+ and CD4+ lymphocytes to the gut was shown in animal studies to be reduced by VDZ. However, blockade of the  $\alpha_4\beta_7$  integrin using a monoclonal antibody that is independent of  $\alpha_4\beta_7$ , etrolizumab, can lead to an even greater reduction in lymphocyte migration. This suggests that although VDZ is effective in reducing lymphocyte migration, other mechanisms of lymphocyte migration independent of  $\alpha_4\beta_7$  are likely active in IBD.(423) In animal models, VDZ has been shown to affect gut migration of Treg cells but it's effect on effector cells is less than that on Treg cells.(424)

It has also been reported that rates of response to VDZ are lower in patients with prior anti-TNF exposure compared to those without prior anti-TNF exposure.(349, 350, 353, 425-427) Therefore there is an unmet need to explore the reasons for treatment failure and to identify potential biomarkers of response and develop new treatments for those failing therapy.

The bowel acts as one of the main interfaces between the external environment and the internal milieu. It maintains a delicate balance between reacting to pathogenic organisms while exhibiting tolerance towards benign commensals, while also allowing for the regulation of salts and the absorption of nutrients and water. As a result, the tissue microenvironment needs significant local controls that are not required by other epithelial surfaces. Epithelial layers, gut associated lymphatic tissue (GALT), and vascular tissues all interact to regulate inflammation in the gut. Examining the local effects of dysregulated inflammation through using human *ex vivo* tissue explants which recapitulate the multicellular tissue microenvironment in inflammatory bowel disease (IBD) will give a greater understanding of disease processes, including mechanisms of drug action and failure.

The advent of multiplex assays now permits detailed assessment of many inflammatory proteins (cytokines, chemokines and growth factors) involved in the gut inflammatory response. Ex-vivo models that permit their detailed assessment within the gut tissue microenvironment are required. Such models could be used to identify biomarkers of response and allow more targeted patient selection for specific therapies.

Patient derived tissue explants have been successfully used in cancer drug development and in biomarker discovery.(428) The use of patient derived tissue explants in the investigation of IBD is a newer development with early studies indicating their potential to aid precision medicine.(401)

Patient derived explants are generated using endoscopically obtained intestinal biopsies that are incubated in suitable media and under conditions favourable to maintaining cell viability. Using UC explants obtained during a scheduled endoscopy could be advantageous compared with serum

sample collection alone. They are representative of the local microenvironment and include multiple cell populations including endothelial, stromal and constituent immune cells. Following incubation of the biopsy in a suitable media, the resulting supernatant, i.e., patient derived explant-conditioned media, can be assessed to detect the presence of secreted proteins and potentially to identify biomarkers of disease outcome. Given current UC treatment algorithms, wherein endoscopic biopsies are an essential component of UC disease assessment, obtaining additional biopsies for explant evaluation could be readily incorporated into routine disease assessment in clinical settings if proven useful in decision making.

Through analysis of secreted proteins, i.e., the cytokines, chemokines and growth factors that are present in the patient derived explant culture medium, the effect of VDZ on the inflammatory proteins within the gut microenvironment can be explored. Additionally, there is the potential to investigate the complex interactions between the intestinal cell populations and immune cells, the peripheral blood mononuclear cells (PBMCs) and to explore how a therapeutic agent such as VDZ might affect their biology.

Peripheral blood mononuclear cells include B and T cells, NK cells, monocytes, and dendritic cells. In donor derived PBMCs the largest cell fractions isolated are naïve or memory T cells. The cellular cross talk and biology of the *ex-vivo* explant model and its secretome can be tested using PBMCs to examine how the VDZ treated secretome of the UC tissue microenvironment might influence cells of the systemic immune system.

The studies in this chapter use such a human *ex vivo* UC explant model to examine the effect of VDZ on the tissue microenvironment, how it affects the inflammatory secretome in the UC gut tissue and how the resulting secretome might influence PBMC biology. To characterise the impact that prior biologic therapy might have on protein secretion profiles in the tissue microenvironment, patients with and without a prior history of biologic agent treatment were also studied.

The overall aim of these studies is to use UC explants to examine the effect that previous biologic therapy might exert on the gut microenvironment, the impact of *ex-vivo* VDZ treatment on inflammatory secretion profiles within the gut microenvironment, and whether the resulting secretome affects PBMC's secretion.

## 4.2 Specific Aims

The three aims in this chapter are:

1. Determine whether prior anti-TNF agent exposure altered the inflammatory secretion profiles in human *ex-vivo* UC explants.
2. Evaluate the effect of *ex-vivo* VDZ treatment on inflammatory secretions from UC explants.
3. Assess if the VDZ treated UC tissue microenvironment and its secretome alter PBMC secretion of IL-15, eotaxin, and eotaxin-3, potentially accounting for the changes in their serum concentrations noted during VDZ induction (see **Chapter 2**)

## 4.3. Methods

### 4.3.1 Study Population

This was a single-centre prospective cohort study. Patients with UC undergoing colonoscopy or sigmoidoscopy for assessment of disease activity were prospectively recruited. Enrolment criteria included patients with an established diagnosis of UC based on clinical, endoscopic, and histological criteria. Patients were ineligible if they were aged under 18 years, unable to give consent, pregnant or had received VDZ.

Baseline demographics and clinical data were obtained by patient questionnaire complemented by medical record review at the time of enrolment. Variables collected included: age, weight, gender, disease duration, 5-aminosalicylate (5-ASA) use, concurrent immunomodulator use (thiopurines/methotrexate), previous biologic therapy exposure (infliximab (IFX), adalimumab, ustekinumab, golimumab), previous small molecule inhibitors (tofacitinib) use, and current corticosteroid therapy. Disease extent and location was classified using the Montreal classification.(336)

Patients were assigned to one of two sequential cohorts based on time of enrolment. Cohort A, referred to as ‘The Anti-TNF Study’, included the first 11 patients enrolled. They participated in the study relating to the impact of prior anti-TNF treatment. Cohort B, referred to as ‘The Vedolizumab Study’, included the next 13 patients. They were included in the *ex vivo* explant VDZ treated study (Figure 4.1).

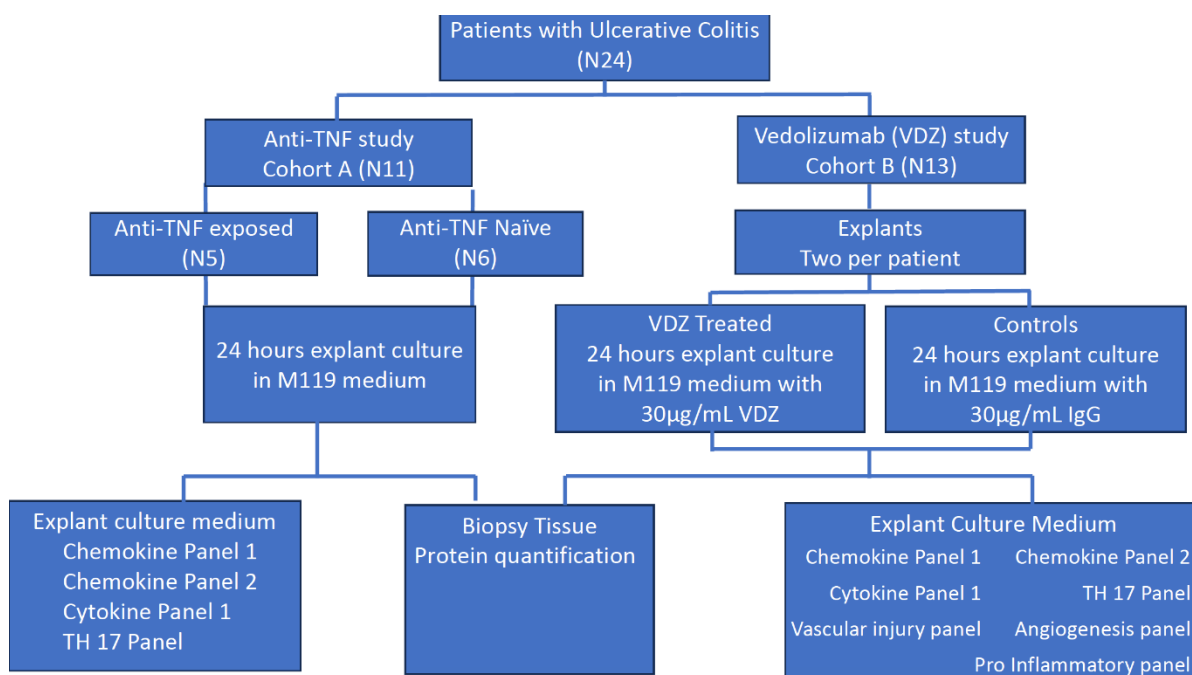


**Figure 4.1. UC Explant Study Flow Diagram**

Twenty-four patients with UC scheduled for endoscopic biopsy and who were receiving UC treatment other than VDZ were recruited. **Cohort A:** 11 patients recruited to the anti-TNF study. Thirty-five inflammatory proteins were measured in UC explant culture media and results of patients with and without prior anti-TNF therapy compared. **Cohort B:** 13 patients recruited to the VDZ study. Fifty-four inflammatory proteins were measured in VDZ treated or IgG treated UC explant culture media.

#### 4.3.2 Ethics and Consent

This project was approved by the Joint St James Hospital and Tallaght University Hospital Research and Ethics Committee SJH/AMNCH (Research Ethics Committee Secretariat Reference Number 2017-04 List 12). Written informed consent was obtained from all patients prior to enrolment.

#### 4.3.3 Endoscopic Examination and Biopsy

Endoscopy was carried out as part of the routine assessment of UC disease activity. Endoscopic assessment was carried out by either a registrar, specialist registrar, advanced nurse practitioner, or consultant gastroenterologist. Two advanced nurse practitioners were present throughout the procedure. Patients were placed in the left lateral position. High-definition endoscopes were used (Eluxeo 700 series colonoscope, Fujifilm™ Tokyo, Japan) with air insufflation. Fentanyl and midazolam conscious sedation was delivered intravenously prior to procedure. Oxygen saturations, heart rate, blood pressure, and respiratory rate were monitored throughout the procedure. UC clinical disease activity was assessed using the partial Mayo sub-score. This, together with the endoscopic Mayo sub-score, combine to form the Mayo score which was developed by Schroeder et al.,(339) and is a recognised validated scoring system.(429) At the end of a scheduled endoscopic procedure, two additional biopsies were obtained in an area of inflammation within 20 centimetres of anal rectal margin. Clinical disease activity was assessed using the partial Mayo subscore

#### 4.3.4 Preparation of Stock Vedolizumab and Immunoglobulin-G

Vedolizumab stocks were prepared in a laminar flow cell culture hood using aseptic techniques. Three hundred milligrams of VDZ (Entyvio®) was reconstituted in 4.8mL of sterile deionized water and dissolved using gentle agitation for 30 minutes. This resulted in a concentration of 60mg/mL which was further diluted in sterile Phosphate Buffered Saline (PBS) to give a sterile stock solution of 600µg/mL VDZ that was divided into 50µL aliquots. The aliquots were stored at -20°C. One milligram native human IgG<sub>1</sub> protein (Abcam No. ab90283) was diluted in sterile PBS to a concentration of 600µg/mL, and separated into 50µL aliquots that were stored at -20°C. These 50µL aliquots were suitable for dilution in 1ml of culture media giving a final concentration of 30µg/mL, which was the final serum concentration obtained during induction in the GEMINI trial.(330)

#### 4.3.5. Ex Vivo Ulcerative Colitis Tissue Explant Culture

Using a technique previously developed by our group to generate tissue explants from patients with IBD,(401) endoscopic biopsy samples were placed on sterile gauze soaked in sterile normal saline and placed in a standard specimen container for immediate transport to the laboratory for

processing. Each biopsy was washed with wash buffer (PBS, Corning, USA), 1% Penicillin/Streptomycin (Gibco, Thermofisher, USA) 1% Fungizone (Sigma-Aldrich, USA) and 0.1% Gentamicin (Lonza, Switzerland). Using a 24 well plate, biopsies were incubated for 24 hours in one millilitre of conditioned media (M119 media (Gibco, Thermofisher, USA), 10% Foetal Bovine Serum (FBS) (Lonza, Switzerland) penicillin (50µg/mL), streptomycin (50µg/mL), and 1µg/mL insulin (Lonza, Switzerland).

For the anti-TNF study, the samples obtained from the first 11 patients, **cohort A**, were categorised into two groups; those with and without a history of prior biologic therapy for later comparison of the patient derived explant conditioned media protein secretion profiles.

In the VDZ study, the biopsies from the subsequent 13 patients, cohort B, were treated with either VDZ or IgG as a control. VDZ at final concentration of 30µg/mL was added to the conditioned medium of one biopsy sample and IgG at a final concentration of 30µg/mL added to the second. Following the addition of VDZ or IgG, the samples were incubated for 24 hours. The concentration of 30µg/mL was selected as this is an equivalent protein concentration to the trough VDZ concentrations that were obtained during induction therapy in the GEMINI trial.(330)

Following the 24-hour incubation, the patient derived tissue explant culture medium was collected and stored at -80°C for subsequent analyses. The matched explant tissue was snap frozen and stored at -80°C until protein was extracted.

#### 4.3.6 Extraction and Quantification of Biopsy Protein Levels

The previously frozen explant tissue was defrosted and added to cryovials with one metal bead and 200µl RadioImmunoPrecipitation Assay buffer (RIPA+buffer) supplemented with EDTA-free protease inhibitor cocktail (Sigma Aldrich, USA) and phosphatase inhibitor (Roche, Switzerland). Biopsy tissue was disrupted using a TissueLyser (Qiagen, Germany) at 25Hz for two minutes. Samples were transferred to clean 1.5mL Eppendorf tubes and centrifuged for 20 minutes at 14,000 RPM at 4°C. In triplicate, 10µL of each sample was added to wells in a 96 well plate (Thermo Pierce BCA microassay, USA) with 200µL of working reagent. The plates were incubated for 30 minutes at

37°C in a humidified CO<sub>2</sub> incubator and using a plate reader, the absorbance at 562nm was determined by subtracting the average absorbance in blank wells from that in wells containing the standard and specimen samples. Protein assay standard curves were prepared to enable determination of the sample protein content.(430)

#### 4.3.7 Determination of Secreted Protein Concentrations in Explant Culture Media

Patient derived tissue explant culture medium was assessed using a V-PLEX Human Biomarker enzyme-linked immunosorbent assay (ELISA) kit (Meso Scale Discovery, Rockville, MD, USA.) to measure the range of analytes. For cohort A, i.e., patients recruited to the anti-TNF study, the conditioned media was assessed for 35 available analytes using Chemokine Panel 1 (eotaxin, eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1 $\alpha$ , MIP-1 $\beta$ , TARC), Chemokine 2 (IL-17A/F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-3, IL-9, TSLP), Cytokine panel 1 (GM-CSF, IL-12/IL-23p40, IL-15, IL-16, IL-17A, IL-1 $\alpha$ , IL-15, IL-7, TNF- $\beta$ , VEGF) and the TH17 panel (IL-17AGenB, IL-21, IL22, IL-23, IL-27, IL-31, MIP-3 $\alpha$ ). As additional panels were available and testing was expanded for cohort B, i.e., those recruited the VDZ study. Fifty-four different analytes were measured in the explant conditioned media derived from patients in Cohort B. In addition to 34 of the 35 analytes listed above (VEGF excluded as it is included in the angiogenesis panel listed below), the vascular injury panel (C-reactive protein (CRP) ICAM-1, SAA, VCAM-1), angiogenesis panel (bFGF, Flt-1, PlGF, Tie-2, VEGF, VEGF-C, VEGF-D), and proinflammatory panel (IFN- $\gamma$ , IL-10, IL-12p70, IL-13, IL-1 $\beta$ , IL-2, IL-4, IL-6, IL-8, TNF- $\alpha$ .) were also used. All assays were run according to the manufacturers guidelines and the results expressed in pg/mL. Undiluted patient derived explant conditioned medium was used for all assays except for the vascular injury panel 2, for which patient derived explant conditioned medium at a one in four dilution was used based on previous studies by our group. The BCA assay (Pierce, the USA) was used to normalise the inflammatory protein secretions to the total protein content in the matched biopsy tissue specimens to generate the final pg/mL for each analyte assessed.

#### 4.3.8 Peripheral Blood Mononuclear Cell Preparation

Peripheral Blood Mononuclear Cells were isolated from a single donor blood pack received from the National Blood Transfusion Laboratory at St. James' Hospital. Cells were isolated using the Ficol

method. In a sterile laminar flow environment, the donor blood was diluted with an equal volume of PBS. This was carefully overlaid onto an equal volume of Lymphoprep™ solution (STEMCELL Technologies, Cambridge, UK) in a sterile 50mL container, taking care not to allow solution mixing. This was centrifuged using a Sorval™ Legend T benchtop centrifuge for 20 minutes at 2000 RMP with deceleration kept to a minimum i.e., no brake applied. Following centrifugation, the white buffy coat layer at the interface between the two solutions was removed using a sterile pasture pipette and centrifuged at 1400 RPM for five minutes with the deceleration brake reapplied. The supernatant was discarded. The residual pellet was re-suspended in PBS, washed, and centrifuged at 1400 RPM three times. The third time the cells were re-suspended in 1640 RPMI medium containing phenol red (a pH indicator), supplemented with 10% foetal bovine serum (FBS) L-glutamine (2mM), penicillin (50µg/mL), and streptomycin (50µg/mL), called complete RPMI solution. This medium was supplemented with 10% foetal bovine serum (FBS) L-glutamine (2mM), penicillin (50µg/mL), and streptomycin (50µg/mL). DMSO at 10v/v% was added to the complete RPMI and snap frozen at -80 °C using Mr. Frosty™ Freezing Container (ThermoFischer) containing isopropyl alcohol.

#### 4.3.9 Peripheral Blood Mononuclear Cell Incubation with Patient Derived Explant Culture Medium

Single donor peripheral blood mononuclear cells were defrosted by the addition 0.5mL of warm (37°C) complete 1640 RPMI solution. The cells were washed three times in sterile PBS and re-suspended in one millilitre of complete RPMI solution and counted using trypan blue in a haemocytometer. Cells at a concentration of  $1.5 \times 10^6$  cells per millilitre were seeded in a 24 well plate. The cells were incubated in a 1:5 dilution of explant conditioned media from the human UC explants. Thirty microlitres of suspended cells with 120µL explant conditioned media that had been treated with IgG or VDZ and incubated in complete RPMI as described above (see **Section 4.3.5**)

All incubations were carried out in a 37°C laminar flow incubator (95%O<sub>2</sub>, 5%CO<sub>2</sub>) with all manipulations carried out under aseptic conditions. Cells were maintained in the 24 well tissue culture plate and incubated for 24 hours, after which they were centrifuged at 1300 RPM in a

Sorvall™ Legend T centrifuge for 20 minutes. The supernatant was collected, transferred to a cryovial and frozen at -80°C for later measurement of the inflammatory proteins; eotaxin, eotaxin-3 and IL-15.

#### 4.3.10 Assessment of Inflammatory Secretions from PBMC's Following Treatment with Tissue Conditioned Media from UC Explants.

Eotaxin Human ELISA Kit (KAC2231), eotaxin-3 Human ELISA Kit (EH171RB), and IL-15 Human ELISA Kit (88-7620-22) (ThermoFisher, USA) were used for ELISA testing. Samples were run according to the manufacturer's protocol. These proteins were chosen for evaluation as in our previous study significant alterations in their serum concentrations had been documented in association with VDZ induction therapy (see Chapter 3).

ELISA wash buffer (1 X PBS, 0.05% Tween) was used for all plate washing and Parafilm® used to seal the plates. Two-fold serial dilutions of the ELISA specific recombinant human proteins (eotaxin, eotaxin-3 and IL-15) with Standard Diluent Buffer containing 7.7 mM sodium azide were used to prepare standard concentrations to generate the standard curve. The standard proteins were diluted two-fold in Reagent Dilutant for six serial dilutions thus enabling generation of a seven-point standard curve from 7.8pg/mL to 500pg/mL for eotaxin and eotaxin-3 and from 19.53pg/mL to 2500pg/mL for IL-15.

All samples were run in duplicate using the standard curves generated using the supplied recombinant ligands. Three ELISA plates were run; each representing one cytokine of interest. To prepare the plates mouse anti-human ligand capture antibody was diluted to a working concentration according to the manufacturer's guideline of which 100µL was added to each well of a 96 well plate, sealed with Parafilm™ and incubated overnight. Following incubation, the wells were thoroughly aspirated, and the plate washed three times. The plate was then blocked using 300µL of incubation buffer, incubated for one hour at room temperature and pressure (RTP). The plate was aspirated and washed three more times.

Following preparation of the plates 100µL of a test sample or standard dilution was added to each well. The plate was again sealed, incubated for two hours, aspirated, and washed three times.

One hundred microlitres of the ELISA specific Detection Antibody, diluted in Reagent Diluent was added to each well. The plate was again sealed, incubated for two hours, washed, and aspirated three times following which 100µL of Streptavidin-HRP (streptavidin conjugated to horseradish peroxidase) was added to each well. This was incubated in the dark for 20 minutes, washed and aspirated three times. One hundred microlitres of Substrate solution (Stabilised Chromogen and Tetramethylbenzidine) (ThermoFisher, USA) was added to each well and incubated for 20 minutes in the dark. Fifty microlitres of stop solution  $\text{NH}_2\text{SO}_4$ , (ThermoFisher, USA) was added and the plate gently agitated.

The optical density of each well was determined using a microplate reader at 450nm and 540nm. Optical imperfection in the plate was corrected by subtracting the reading at 540nm from readings at 450nm.

#### 4.3.11 Statistical Analysis

Descriptive statistics were applied to the baseline demographics with continuous variables presented with median and inter quartile range [IQR] and categorical variables summarised as percentages. The Mann Whitney U test was used to compare categorical and continuous independent data between groups by prior biologic exposure history. Paired continuous data were interpreted using the Wilcoxon sign-rank test. A False Discovery Rate (FDR) control was not used in the primary statistical analysis and a  $p < 0.05$  was deemed significant. However, in the secondary multivariate analysis to guard against Type 1 error (false positives) a FDR control using the Benjamini–Hochberg procedure for multiple test correction with the FDR of 5% assigned was applied. As per standard protocol, the largest p value that was less than the Benjamini–Hochberg critical value was identified. All comparisons with a p value less than or equal to this threshold p value were considered significant following FDR control. All statistical analyses were performed

using SPSS (Version 26.1.2; IBM, NY, USA). Graphs were constructed using GraphPad Prism (version 5.01: GraphPad Software Inc.).

## 4.4 Results

### 4.4.1 Patient and Disease Characteristics

Twenty-four patients, fifteen males and nine females with UC scheduled for endoscopic assessment were recruited. The demographics and clinical characteristics of the 24 patients, 11 in cohort A and 13 in cohort B are summarised in **Table 4.1**. In these cohorts, anti-TNF agents were the only biologic therapies patients received by any patient. Fifty-four percent of patients had received no prior biologic therapy.

Eleven patients, **Cohort A**, participated in the study to assess the effect of prior anti-TNF exposure on inflammatory protein secretions from the UC tissue microenvironment. Their median age at time of endoscopy was 54.7 years with an almost equal numbers of male and female patients. No patients had proctitis, five had extensive colitis, and six had left sided colitis. Disease was of mild to moderate severity overall with a mean baseline partial Mayo sub-score of six. No patient had a partial Mayo sub-score of zero. Five patients were biologic therapy naïve. Six had had prior anti-TNF therapy. No patient had prior exposure to VDZ, ustekinumab, JAK-STAT inhibitors or biologic therapy other than anti-TNF therapy.

The next 13 patients recruited, **Cohort B**, were included in the VDZ treated *ex vivo* explant study and explant conditioned media derived from this cohort were also used in the PBMC study.

The demographics and clinical characteristics of this cohort are listed with no significant differences identified between the cohorts (**Table 4.1**). The median age of 13 patients, nine males and four females, at time of endoscopy was 40.5 years. One patient had proctitis, four had extensive colitis, and eight had left sided colitis. Disease was of mild to moderate severity overall with a mean baseline partial Mayo sub-score of three. Eight patients were biologic therapy naïve, five had had prior anti-TNF therapy, and no patient had prior exposure to VDZ, ustekinumab, JAK-STAT inhibitors, or biologic therapy other than anti-TNF agents.

|                                                                                                         | All Patients<br>N24                 | Anti-TNF study<br>Cohort A<br>N11  | VDZ study<br>Cohort B<br>N13       | p Value*                      |
|---------------------------------------------------------------------------------------------------------|-------------------------------------|------------------------------------|------------------------------------|-------------------------------|
| <b>Age in years</b><br><b>Median</b><br><b>[range]</b>                                                  | 42.5<br>[22.6-75.6]                 | 54.7<br>[35.1-75.6]                | 40.5<br>[22.6-75.4]                | p=0.15                        |
| <b>Gender</b><br>Female<br>Male                                                                         | 9 (38%)<br>15 (63%)                 | 5 (45%)<br>6 (55%)                 | 4 (31%)<br>9 (69%)                 | p=0.459                       |
| <b>Disease Duration (years)</b><br><b>Median [range]</b>                                                | 7.4[0.8-32.7]                       | 11.5 [0.9–32.7]                    | 5.4 [0.8–14.5]                     | p=0.106                       |
| <b>Disease Extent UC</b><br>Proctitis<br>Left-sided colitis<br>Extensive colitis                        | 1 (4%)<br>14 (58%)<br>9 (38%)       | 0<br>6 (55%)<br>5 (45%)            | 1 (8%)<br>8 (62%)<br>4 (31%)       | p=0.883<br>p=0.120<br>p=0.548 |
| <b>Endoscopic Mayo Subscore</b><br><b>Partial Mayo Subscore</b>                                         | 2 [1-3]<br>5 [0–9]                  | 2 [1–3]<br>6 [2–9]                 | 2 [1–3]<br>3 [0–8]                 | p=0.494<br>p=0.018            |
| <b>5-Aminosalicylates</b>                                                                               | 20 (83%)                            | 9 (82%)                            | 11 (85%)                           | p=0.855                       |
| <b>Corticosteroids</b>                                                                                  | 5 (21%)                             | 4 (36%)                            | 1 (8%)                             | p=0.085                       |
| <b>Immunomodulators</b><br>Thiopurines<br>Methotrexate                                                  | 4<br>0                              | 1<br>0                             | 3<br>0                             | p=0.36                        |
| <b>Prior Anti-TNF Therapy</b><br>Naïve<br>≥1 previous agent<br>≥2 previous agents<br>≥3 previous agents | 13 (54%)<br>9 (38%)<br>4 (17%)<br>0 | 5 (45%)<br>4 (36%)<br>2 (18%)<br>0 | 8 (62%)<br>5 (38%)<br>2 (15%)<br>0 | p=0.621<br>p=0.916<br>p=0.855 |

Categorical variables are presented as percentages; in some instances the sum of the percentages exceed 100% due to rounding. Continuous variables are presented as median and range.

\*p values to compare cohorts A and B were calculated using the Mann Whitney U for continuous variables and Chi squared test for categorical variables

**Table 4.1. Demographics and Clinical Characteristics of 24 patients with Ulcerative Colitis**

Demographic and clinical characteristics of 24 patients with UC at time of study enrolment; 11 in the anti-TNF study (Cohort A) and 13 in the VDZ study (Cohort B). Both cohorts were similar with no significant differences in characteristics between the cohorts identified.

#### 4.4.2 Association of Inflammatory Protein Secretions in Ulcerative Colitis Patients with and without Prior Anti-TNF Exposure

Thirty-five different analytes; eotaxin, eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1 $\alpha$ , MIP-1 $\beta$ , TARC, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-3, IL-9, TSLP, GM-CSF, IL-12/IL-23p40, IL-15, IL-16, IL-17A, IL-1 $\alpha$ , IL-15, IL-7, TNF- $\beta$ , VEGF, IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, and MIP-3 $\alpha$  were measured in patient derived explant conditioned media. The results obtained relating to patients previously treated with a biologic agent, an anti-TNF agent, and those naïve to such treatment were compared. The only biologic therapy that patients in this cohort had received was anti-TNF inhibitor therapy. These 35 inflammatory proteins were selected for testing based on their roles in host defence, regulation of inflammatory pathways, and platform availability. Numeric differences between the two groups for individual analytes were observed, but fell short of the threshold for statistical significance (**Table 4.2**). The overall concentrations of these 35 analytes in the UC tissue explant conditioned media were similar in patients with and without anti-TNF therapy.

| Serum Cytokine Concentrations (pg/mL), Median [IQR] |                |                     |                        |                     |         |
|-----------------------------------------------------|----------------|---------------------|------------------------|---------------------|---------|
|                                                     | Anti TNF Naïve |                     | Prior Anti TNF therapy |                     | p Value |
| Chemokine Panel 1                                   |                |                     |                        |                     |         |
| Eotaxin                                             | 5.78           | [3.94-6.10]         | 11.66                  | [6.72-15.47]        | NS      |
| Eotaxin-3                                           | 13.99          | [5.68-18.42]        | 16.85                  | [8.86-25.97]        | NS      |
| IL-8                                                | 25473.25       | [25052.77-32948.12] | 42420.67               | [28146.80-61277.35] | NS      |
| IP-10                                               | 20.88          | [19.11-26.18]       | 14.79                  | [13.30-17.45]       | NS      |
| MCP-1                                               | 161.76         | [61.47-271.20]      | 355.32                 | [249.43-505.02]     | NS      |
| MCP-4                                               | 9.796          | [6.98-15.40]        | 16.92                  | [10.54-18.05]       | NS      |
| MDC                                                 | 215.20         | [16.03-247.68]      | 248.33                 | [228.60-264.32]     | NS      |
| MIP-1α                                              | 316.21         | [27.62-333.72]      | 165.23                 | [102.60-515.53]     | NS      |
| MIP-1β                                              | 150.36         | [56.80-243.66]      | 131.26                 | [87.65-302.53]      | NS      |
| TARC                                                | 4.59           | [2.05-5.26]         | 4.85                   | [2.94-5.40]         | NS      |
| Chemokine Panel 2                                   |                |                     |                        |                     |         |
| IL-17A/F                                            | 3.06           | [0.95-8.57]         | 2.69                   | [1.71-3.76]         | NS      |
| IL-17B                                              | 1.06           | [0.95-1.96]         | 1.83                   | [1.29-2.54]         | NS      |
| IL-17C                                              | 10.44          | [5.16-25.39]        | 10.55                  | [9.83-13.13]        | NS      |
| IL-17D                                              | 5.29           | [4.43-9.48]         | 8.55                   | [5.91-25.00]        | NS      |
| IL-1RA                                              | 1177.60        | [1011.46-1245.60]   | 1294.22                | [1041.10-1396.15]   | NS      |
| IL-3                                                | 9.10           | [2.46-9.28]         | 1.79                   | [0.26-4.78]         | NS      |
| IL-9                                                | 0              | [0.00-0.00]         | 0                      | [0.00-0.00]         | NS      |
| TSLP                                                | 15.03          | [1.70-23.86]        | 8.46                   | [6.09-12.81]        | NS      |
| Cytokine Panel 1                                    |                |                     |                        |                     |         |
| GM-CSF                                              | 396.23         | [64.47-684.39]      | 363.72                 | [124.75-501.52]     | NS      |
| IL-12/IL-23p40                                      | 7.13           | [0.49-18.65]        | 11.08                  | [9.63-13.90]        | NS      |
| IL-15                                               | 0.66           | [0.26-0.69]         | 0.54                   | [0.49-0.75]         | NS      |
| IL-16                                               | 644.20         | [311.11-1130.09]    | 977.25                 | [933.50-1123.43]    | NS      |
| IL-17A                                              | 20.16          | [13.61-72.94]       | 27.34                  | [24.32-30.43]       | NS      |
| IL-1α                                               | 36.44          | [25.60-58.11]       | 45.47                  | [21.83-51.35]       | NS      |
| IL-5                                                | 0.19           | [0.09-0.47]         | 0.18                   | [0.10-0.53]         | NS      |
| IL-7                                                | 0.29           | [0.13-0.62]         | 0.98                   | [0.27-1.61]         | NS      |
| TNF-β                                               | 0.28           | [0.09-0.40]         | 0.21                   | [0.17-0.25]         | NS      |
| VEGF                                                | 271.37         | [238.15-459.86]     | 443.80                 | [204.95-746.59]     | NS      |
| TH 17 Panel                                         |                |                     |                        |                     |         |
| IL-17A GenB                                         | 12.99          | [7.75-44.62]        | 16.95                  | [14.05-19.38]       | NS      |
| IL-21                                               | 1.22           | [1.04-1.44]         | 0.93                   | [0.48-1.19]         | NS      |
| IL-22                                               | 75.48          | [5.86-374.53]       | 34.57                  | [18.46-40.98]       | NS      |
| IL-23                                               | 7.25           | [2.18-12.87]        | 10.18                  | [9.65-11.38]        | NS      |
| IL-27                                               | 26.59          | [24.82-38.00]       | 37.08                  | [20.47-53.89]       | NS      |
| IL-31                                               | 0.23           | [0.18-0.27]         | 0.20                   | [0.15-0.29]         | NS      |
| MIP-3α                                              | 43.66          | [13.28-63.74]       | 19.85                  | [17.39-41.30]       | NS      |

\*Secreted protein concentrations (pg/mL per  $\mu$ g protein) are presented as Median and InterQuartile range [IQR]

\*\*For all comparison the Mann-Whitney *U* test was applied

\*\*\* The False Discovery Rate (FDR) was controlled using the Benjamini–Hochberg procedure for multiple test correction with the FDR assigned as 5%. The threshold of significance for 54 analytes was  $p = 0.0046$

**Table 4.2. Concentrations, median [IQR], of 35 Inflammatory Proteins in UC Explant conditioned media from Patients with and Without Prior Anti-TNF Therapy.**

Comparison of 35 inflammatory proteins in UC explant tissue conditioned media derived from patients naïve to anti-TNF agents and those with a history of prior anti-TNF therapy. In univariate analysis no statistically significant differences were found.

#### 4.4.3 Patient Derived Explant Culture Medium Protein Secretion Following Vedolizumab Incubation

The concentrations of three of 54 inflammatory proteins, Interferon- $\gamma$ -inducible protein (IP)-10, Macrophage Derived Chemokine (MDC), and InterLeukin (IL)-21, were significantly altered in VDZ treated UC explant conditioned media compared with the IgG treated control (**Figure 4.2**).

IP-10, median[IQR], was lower in VDZ treated conditioned media, 15.09pg/mL [4.65-35.59pg/mL], compared with the IgG controls, 22.03pg/mL [4.34-102.11pg/mL],  $p=0.016$ . Similarly, MDC at 250.8pg/mL [127.49-623.69pg/mL] was lower in VDZ treated conditioned media than in the controls, 412.15pg/mL [139.74-1356.46pg/mL],  $p=0.039$  as was IL-21 was 0.00pg/mL [0.00-0.96pg/mL] compared with 1.17pg/mL [0.00-2.61pg/mL],  $p=0.05$  (**Table 4.3**). However, when the Benjamini–Hochberg procedure for multiple test correction was applied with the FDR assigned as 5%, the threshold of significance for 54 analytes was  $p= 0.0046$ . No analyte reached threshold of significance following multiple test correction.

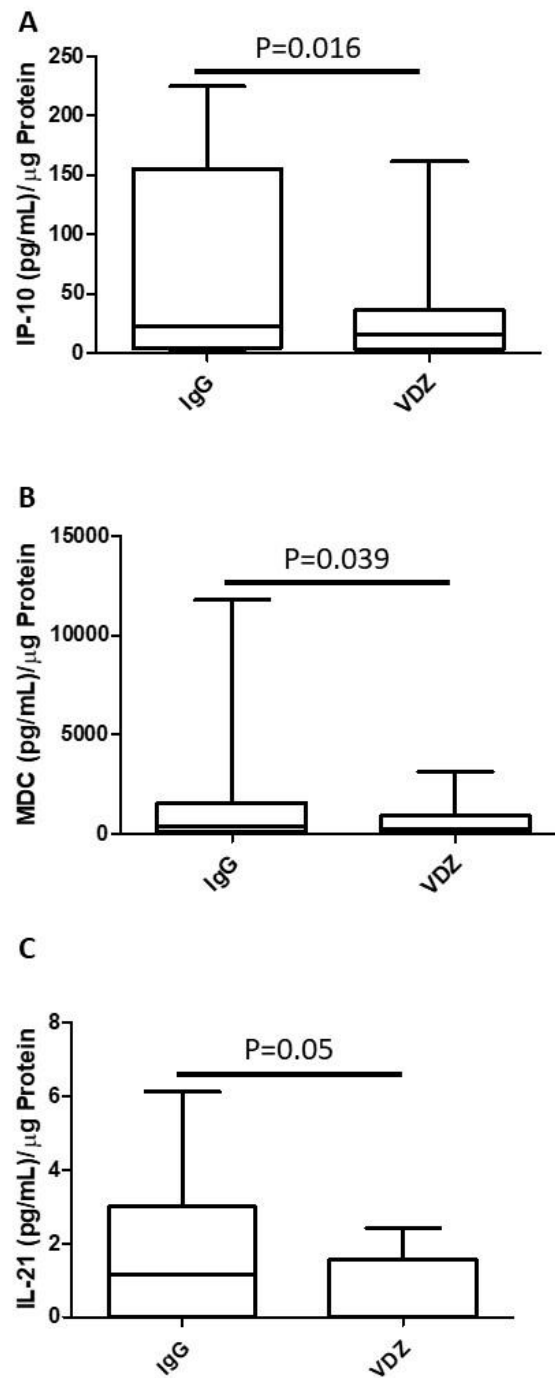


**Figure 4.2** Boxplots [median, IQR, range] of IP-10, MDC and IL-21 concentrations in conditioned media of VDZ Treated UC Explants Compared with IgG Treated Controls

In univariate analysis the concentrations of three cytokines; **A)** Interferon- $\gamma$ -inducible protein (IP)–10 **B)** Macrophage Derived Chemokine (MDC) and **C)** IL-21 were significantly lower in VDZ treated explant conditioned media compared with IgG treated controls. For all comparison the Wilcoxon Signed rank test was applied. However, when the Benjamini–Hochberg procedure for multiple test correction was applied the threshold of significance was not met.

| Cytokine Concentrations (pg/mL), Median [IQR]<br>in patient derived explant conditioned media treated with IgG (control) or vedolizumab |                     |                     |                     |                     |         |
|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------|---------------------|---------------------|---------------------|---------|
|                                                                                                                                         | Control IgG 30µg/mL |                     | Vedolizumab 30µg/mL |                     | P Value |
| Angiogenesis Panel                                                                                                                      |                     |                     |                     |                     |         |
| bFGF                                                                                                                                    | 355.35              | [157.14-500.26]     | 335.37              | [122.67-465.32]     | NS      |
| Flt-1                                                                                                                                   | 314.20              | [113.44-440.22]     | 172.58              | [65.11-361.40]      | NS      |
| PlGF                                                                                                                                    | 22.97               | [16.44-32.64]       | 10.55               | [6.51-19.79]        | NS      |
| Tie-2                                                                                                                                   | 0.00                | [0.00-0.00]         | 0.00                | [0.00-0.00]         | NS      |
| VEGF                                                                                                                                    | 527.21              | [321.46-1466.76]    | 553.75              | [300.97-1504.74]    | NS      |
| VEGF-C                                                                                                                                  | 0.00                | [0.00-0.00]         | 0.00                | [0.00-0.00]         | NS      |
| VEGF-D                                                                                                                                  | 0.00                | [0.00-0.00]         | 0.00                | [0.00-0.00]         | NS      |
| Chemokine Panel 1                                                                                                                       |                     |                     |                     |                     |         |
| Eotaxin                                                                                                                                 | 19.17               | [13.78-32.81]       | 19.73               | [14.04-30.81]       | NS      |
| Eotaxin-3                                                                                                                               | 64.78               | [46.14-82.34]       | 51.56               | [38.35-72.10]       | NS      |
| IL-8                                                                                                                                    | 59617.35            | [31989.20-81826.17] | 39407.19            | [32175.83-99653.72] | NS      |
| IP-10                                                                                                                                   | 22.03               | [4.34-102.11]       | 15.09               | [4.65-35.59]        | p=0.016 |
| MCP-1                                                                                                                                   | 547.92              | [280.43-1415.91]    | 236.52              | [120.90-985.99]     | NS      |
| MCP-4                                                                                                                                   | 38.48               | [26.11 - 61.78]     | 45.77               | [27.17-66.67]       | NS      |
| MDC                                                                                                                                     | 412.15              | [139.74-1356.46]    | 250.8               | [127.49-623.69]     | p=0.039 |
| MIP-1α                                                                                                                                  | 493.17              | [85.62-3082.90]     | 0                   | [0.00–0.00]         | NS      |
| MIP-1β                                                                                                                                  | 286.61              | [59.95-868.69]      | 378.72              | [86.43-743.03]      | NS      |
| TARC                                                                                                                                    | 9.15                | [3.50-14.87]        | 9.41                | [4.08-12.68]        | NS      |
| Chemokine Panel 2                                                                                                                       |                     |                     |                     |                     |         |
| IL-17A/F                                                                                                                                | 6.72                | [3.77-21.02]        | 4.82                | [2.73-7.42]         | NS      |
| IL-17B                                                                                                                                  | 4.96                | [1.32-10.56]        | 2.51                | [1.14-6.56]         | NS      |
| IL-17C                                                                                                                                  | 12.07               | [3.95-23.36]        | 7.04                | [1.69-18.14]        | NS      |
| IL-17D                                                                                                                                  | 9.83                | [5.82-19.92]        | 7.87                | [4.57-20.07]        | NS      |
| IL-1RA                                                                                                                                  | 3143.56             | [1485.46-3802.75]   | 2632.29             | [1544.35-3752.06]   | NS      |
| IL-3                                                                                                                                    | 17.08               | [13.73-50.33]       | 14.96               | [9.24-37.69]        | NS      |
| IL-9                                                                                                                                    | 0.00                | [0.00-0.31]         | 0.05                | [0.00-0.61]         | NS      |
| TSLP                                                                                                                                    | 13.83               | [4.99-28.89]        | 8.75                | [3.50-28.42]        | NS      |
| Cytokine Panel 1                                                                                                                        |                     |                     |                     |                     |         |
| GM-CSF                                                                                                                                  | 1050.04             | [132.41-2481.82]    | 1185.07             | [377.94-1571.53]    | NS      |
| IL-12/IL-23p40                                                                                                                          | 13.79               | [6.03-41.99]        | 12.12               | [3.81-22.83]        | NS      |
| IL-15                                                                                                                                   | 1.13                | [0.61-2.08]         | 0.82                | [0.62-1.37]         | NS      |
| IL-16                                                                                                                                   | 1737.59             | [1143.66-3939.71]   | 1071.43             | [666.92-2160.38]    | NS      |
| IL-17A                                                                                                                                  | 40.79               | [20.02-94.33]       | 27.26               | [18.74-52.38]       | NS      |
| IL-1α                                                                                                                                   | 165.22              | [22.71-260.49]      | 124.61              | [29.49-294.63]      | NS      |
| IL-5                                                                                                                                    | 1.02                | [0.41-2.59]         | 0.67                | [0.27-0.83]         | NS      |
| IL-7                                                                                                                                    | 0.58                | [0.42-2.68]         | 0.53                | [0.31-1.07]         | NS      |
| TNF-β                                                                                                                                   | 0.00                | [0.00–0.00]         | 0.00                | [0.00–0.00]         | NS      |

| Cytokine Concentrations (pg/mL), Median [IQR]<br>in patient derived explant conditioned media treated with IgG (control) or vedolizumab |                     |                   |                     |                   |         |
|-----------------------------------------------------------------------------------------------------------------------------------------|---------------------|-------------------|---------------------|-------------------|---------|
|                                                                                                                                         | Control IgG 30µg/mL |                   | Vedolizumab 30µg/mL |                   | P Value |
| Proinflammatory Cytokine Panel                                                                                                          |                     |                   |                     |                   |         |
| IFN-γ                                                                                                                                   | 77.78               | [45.05-127.22]    | 52.53               | [37.56-82.82]     | NS      |
| IL-10                                                                                                                                   | 1215.61             | [923.71-3376.01]  | 1234.96             | [883.94-3360.02]  | NS      |
| IL-12p70                                                                                                                                | 22.68               | [9.02-35.44]      | 17.06               | [6.12-30.56]      | NS      |
| IL-13                                                                                                                                   | 56.57               | [27.05-71.27]     | 52.18               | [24.22-65.79]     | NS      |
| IL-1β                                                                                                                                   | 51.96               | [25.30-151.78]    | 75.83               | [34.14-183.29]    | NS      |
| IL-2                                                                                                                                    | 10.20               | [8.40-24.96]      | 11.14               | [7.33-26.22]      | NS      |
| IL-4                                                                                                                                    | 21.27               | [15.43-27.64]     | 20.94               | [12.80-28.10]     | NS      |
| IL-6                                                                                                                                    | 2339.87             | [1549.80-3877.24] | 2165.82             | [1489.50-3502.32] | NS      |
| IL-8                                                                                                                                    | 2084.99             | [0.72-3105.90]    | 2314.19             | [1656.29-2674.05] | NS      |
| TNF-α                                                                                                                                   | 26.67               | [15.93-63.76]     | 26.88               | [10.01-54.86]     | NS      |
| TH 17 Panel                                                                                                                             |                     |                   |                     |                   |         |
| IL-17A<br>GenB                                                                                                                          | 0.06                | [0.00-4.19]       | 0.00                | [0.00-0.27]       | NS      |
| IL-21                                                                                                                                   | 1.17                | [0.00-2.61]       | 0.00                | [0.00-0.96]       | p=0.05  |
| IL-22                                                                                                                                   | 28.75               | [1.73-405.37]     | 44.05               | [0.97-151.54]     | NS      |
| IL-23                                                                                                                                   | 11.09               | [0.00-31.14]      | 3.13                | [0.00-22.29]      | NS      |
| IL-27                                                                                                                                   | 87.86               | [0.08-127.03]     | 62.37               | [0.07-98.42]      | NS      |
| IL-31                                                                                                                                   | 0.17                | [0.00-0.64]       | 0.08                | [0.00-0.39]       | NS      |
| MIP-3α                                                                                                                                  | 44.65               | [0.41-111.64]     | 31.42               | [0.19-83.32]      | NS      |
| Vascular Injury Panel 1                                                                                                                 |                     |                   |                     |                   |         |
| CRP                                                                                                                                     | 432.24              | [107.01-1256.13]  | 313.16              | [93.74-798.85]    | NS      |
| ICAM-1                                                                                                                                  | 5883.85             | [4106.82-9779.22] | 5480.23             | [3542.97-8174.74] | NS      |
| SAA                                                                                                                                     | 306.46              | [259.78-477.40]   | 262.01              | [163.84-659.55]   | NS      |
| VCAM-1                                                                                                                                  | 4041.51             | [3389.26-5973.53] | 3482.27             | [2862.93-4695.97] | NS      |

\*Secreted protein concentrations (pg/mL per µg protein) are presented as median and InterQuartile range [IQR]

\*\*For all comparison the Wilcoxon Signed rank test was applied.

**Table 4.3. Concentrations, median [IQR], of 54 inflammatory proteins in UC Explant conditioned media following treatment with vedolizumab or with IgG (control)**

#### 4.4.4 The Effect of Vedolizumab Incubation on Eotaxin, Eotaxin-3, and IL-15 Secretion by PBMCs

In our previous study serum levels of eotaxin, and eotaxin-3 were significantly higher and IL-15 significantly lower following VDZ induction compared with baseline,  $p=0.0012$ ,  $p=0.0013$ ,  $p=0.0004$  respectively (see **Chapter 3**). To determine if PBMC secretion of these proteins might be affected by the gut tissue secretome following VDZ treatment, these three analytes were measured in PBMC supernatant after PBMC incubation with either the VDZ treated or IgG treated UC explant conditioned media and the results compared.

Eotaxin and eotaxin 3 were not detected by ELISA in any of the tested supernatants. The concentration of IL-15 in the supernatant retrieved from PBMCs incubated with the VDZ treated UC explant conditioned media was significantly lower, 202pg/mL [IQR 93–209pg/mL] compared with that from the PBMCs incubated with the IgG treated UC explant conditioned media, 215pg/mL [IQR 208–227pg/mL], (**Figure 4.3**).

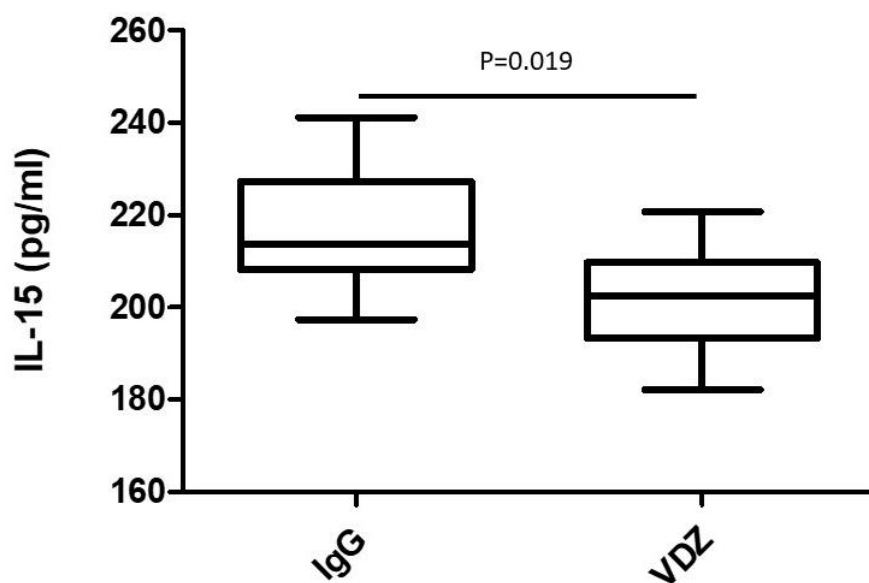


**Figure 4.3. Boxplots [median, IQR, range] of IL-15 concentration in supernatant of PBMCs following incubation with either VDZ treated or IgG treated UC explant conditioned media.** Lower concentrations of IL-15 were found in the supernatant of PBMCs stimulated with the VDZ treated explant culture medium compared to the IgG treated control. For all comparison the Wilcoxon Signed rank test was applied.

#### 4.5 Strengths and Limitations

In these studies I examined how VDZ, a novel therapeutic agent, might exert its effect on the local gut tissue microenvironment in patients with UC. Furthermore, using UC explants I studied the interaction of the local VDZ treated microenvironment and its secretome with healthy donor PBMC's as this could be one pathway for cross-talk between the gut and peripheral immune cells.

The strengths of the study include that the patient cohort is well characterised and the data is complete. Based on the results relating to Cohort A, that prior anti-TNF therapy did not alter the expression of the tested analytes and that there were no significant differences in patient characteristics between the two cohorts, A and B., it is reasonable to assume that the findings in cohort B are generalisable to IBD patients irrespective of whether or not they had received anti-TNF therapy.

While a definite biomarker of VDZ response was not determined, nonetheless these studies demonstrate how the *ex vivo* explant model can be used to help define the role of inflammatory proteins in IBD. It provides a way to investigate how therapeutic agents modulate the inflammatory process using a three dimensional model, without tissue and cellular dissociation, maintaining spatial arrangement to keep cellular signalling pathways and cellular communications intact.

Study limitations include the relatively small numbers of patients in each cohort. This increases the possibility of a Type II error, inherently reducing the likelihood that statistical testing will yield a significant result particularly when a FDR correction is applied. There is a risk that relevant changes could be dismissed thus due consideration must be given to those changes identified in univariate analysis that may just fall short of attaining significance when correction for multiplicity is applied.

A potential study limitation is that biopsies were not assessed for cellular composition. Vedolizumab's known mechanism of action is to bind the  $\alpha_4\beta_7$  integrin expressed on lymphocytes. It is also thought that VDZ may exert an effect on the innate system through alteration of macrophage cytokine expression.(378) Variation in the cellular composition of the explants and the relative proportions of the different cell types could vary the response to VDZ. Characterisation of

the biopsies' cellular populations would help us to understand changes in the secretome and why inflammatory protein content in the secretome can differ from that in serum.

The results presented relate to an *ex-vivo* model with *in-vitro* incubation of the UC explant with VDZ. Using the explant model I have shown the feasibility of measuring inflammatory proteins in UC tissue. It would be of interest to extend these studies and compare these findings with those using explants from patients established on VDZ therapy; those in remission and those failing therapy. Using explants from patients undergoing treatment brings the model a step closer to the real world experience of patients responding or failing VDZ therapy. Characterising the differences between remitting and non-remitting patients might provide more clarity as to why some fail and identify biomarkers of response. If equivalence in results derived from UC explants incubated with VDZ *in vitro*, as in this study, with those obtained from explants derived from patients receiving VDZ were established this would provide validation of the model as truly reflective of IBD microenvironment and potentially validate it as a reliable model for assessing the impact of novel agents on local inflammatory pathways in IBD.

Reasons for failure of anti-TNF therapy, primary non-response, secondary loss of response, or intolerance were not considered in this study. The time from anti-TNF exposure to biopsy or time on anti-TNF therapy was not captured.

The PBMCs were not identified as the source of eotaxin or eotaxin-3 in this study, however the PBMCs were derived from a healthy donor rather than a patient with active UC. While this is unlikely to affect the results, future studies using PBMCs obtained from the study patients and characterised by disease status, remitting or non-remitting, would be of great interest.

#### 4.6 Discussion

Prior anti-TNF therapy had no apparent effect on a broad range of inflammatory proteins in UC-explant culture medium. In univariate analysis no significant differences were found between the concentrations of 35 inflammatory proteins in UC explants derived from patients who had prior anti-TNF therapy compared with those derived from patients who were anti-TNF naïve.

Anti-TNF therapy has significant effects on the tissue microenvironment. It leads to decreased levels of TNF- $\alpha$  and IFN- $\gamma$ ,<sup>(431)</sup> decreased levels of adaptive immune cells,<sup>(432)</sup> and decreased expression of genes involved in angiogenesis.<sup>(433)</sup> It significantly upregulate mucosal expression of IL23-p19 subunit, IL23 receptor and IL-17A.<sup>(434)</sup>

Anti-TNF therapy may exhibit an effect by not just removing soluble TNF- $\alpha$  from circulation but also by binding membrane bound TNF and blocking TNF/TNFR2 receptor complex and causing T-cell apoptosis. CD14<sup>+</sup> macrophages express IL-23R as well as TNFR2. It has been suggested that the blockage of the TNF/TNFR2 pathway may result in pressure on alternative pathways of inflammation e.g., with increased expression of IL23R and increase in TNF- $\alpha$  independent pathways of inflammation.<sup>(434)</sup> This might be viewed as analogous to the selective pressure exerted by antibiotics that can drive emergence of antibiotic resistance. We aimed to see if anti-TNF therapy and the resulting change in the inflammatory profiles might have lasting effects within the tissue micro-environment.

A subsequent study from our group with higher patient numbers that allowed for comparison of those with and without anti-TNF failure identified anti-TNF failure as a potential biomarker of VDZ treatment failure.<sup>(401)</sup> In that study five patients with documented anti-TNF failure were compared to 19 patients with no history of failure; significant differences in the patient derived explant conditioned media concentrations of 14 cytokines were found. These included cytokines involved in IL-23 pathway including the p40 subunit of IL-12/23 and IL-17. In other studies patients who respond to anti-TNF therapy have been shown to have higher concentrations of TNFR2 receptor. During anti-TNF therapy there is significant up regulation of mucosal expression IL-23-p19 subunit, IL-23 receptor and IL-17A.<sup>(434)</sup>

Anti-TNF therapy can affect colonic tissue with higher levels of VEGF and ANG2 found in IBD patients when compared to controls. ANG1 also is significantly affected by anti-TNF therapy; higher baseline levels of VEGF and ANG1 are associated with week 14 remission.<sup>(433)</sup>

Studies have indicated that prior anti-TNF therapy for IBD has been associated with VDZ treatment failure, the reasons for this are not evident in our study. It may be that patients with anti-TNF failure have a more severe inflammatory phenotype that is not evident in the range of inflammatory proteins analysed in this study.

As noted above, no differences in concentrations of tested inflammatory proteins in the secretome of those with and without a history of prior anti-TNF therapy were found, however this could indicate that our study was under powered. Anti-TNF failure is multifactorial; it may be due to primary non-response, secondary non-response, or intolerance. Our study with low numbers of patients with TNF exposure did not address other potential causes.

Since completion of this work, using the same methodology, UC colonic patient derived explants have been successfully used in our laboratory to examine the association between the gut microenvironment and UC disease status.(401) The studies reported here were important in providing a proof of concept for such further investigations. The concepts explored in these studies and now been extended by others represent important steps toward development of precision medicine for patients with IBD.

In UC, the response to VDZ induction therapy can take up to 12 weeks to manifest. Our study shows that after 24 hours incubation with colonic tissue, VDZ begins to exert an effect on inflammatory protein secretion in the gut microenvironment. We show that incubation of outpatient derived UC explants with VDZ resulted in a reduction in the secretion of IP-10, MDC, and IL-21, proteins that are pro-inflammatory and elevation of which has been associated with IBD(435-441)

The effect of VDZ induction on a broad range of serum inflammatory proteins *in vivo* has been reported in a previous chapter (see **Chapter 3**). However, there is a paucity of data relating to the effect of VDZ within the tissue microenvironment. In our UC explant study of 13 patients, published as a conference poster, UC explants treated with VDZ compared with an IgG control showed that in univariate analysis GM-CSF, IL-16, IL-22, IL-23, and V-CAM concentrations were significantly

reduced,(442, 443) however correction for multiplicity was not reported. Our study did not find significant changes in these biomarkers following statistical correction for multiplicity.

Petito et al., reported finding lower concentration of IFN- $\gamma$ , TNF-alpha, IL-17, IL-6 and IL-8 following IFX treatment of UC explants (444), while in other studies IFX has been shown to upregulate IL-25 expression.(445)

Interferon  $\gamma$  Induced Protein (IP-10) also known as CXCL is one of the cytokines found to be affected by VDZ in our study. IP-10 is secreted by T lymphocytes, neutrophils, monocytes, fibroblasts, and endothelial cells in response to IFN- $\gamma$ . It binds the chemokine receptor CXCR3 leading to lymphocyte migration and differentiation of lymphocytes from naïve to pro inflammatory cells. Increased concentrations of IP-10 are found in patients with UC and CD compared with the general population.(446, 447). Higher levels of IP-10 are found in inflamed colonic tissue when compared to adjacent non inflamed tissue in UC.(448). Serum multiplex studies have revealed an association between IP-10 and an increased frequency of extraintestinal IBD manifestations. In mouse models of Crohn's disease anti-IP-10 was shown to reduce histological disease activity.(440, 449) It was considered such a promising IBD treatment target that a specific anti-IP-10 biologic agent was developed. Eldelumab was a monoclonal antibody developed to treat IBD by targeting IP-10. However, in phase II randomised controlled trials, eldelumab proved unsuccessful in inducing remission in patients with UC(438) or CD.(450) VDZ primarily acts through inhibition of  $\alpha_4\beta_7$  integrin to block lymphocyte migration through blood vessels into the gut. The reduction in IP-10 in patient derived explant culture medium detected in our study raises the possibility that it may also exerts an effect in the tissue micro-environment that leads to reduction of IP-10 and its pro-inflammatory activity.

Macrophage Derived Chemokine (MDC) also known as CCL22, first described in 1997,(451) is expressed by macrophages and dendritic cells. It functions as a chemoattractant for TH2 cells, NK cells, and monocytes. Elevated levels of MDC is associated with inflammatory conditions including atopic dermatitis,(452) and atherosclerosis.(453) Serum concentrations of MDC and eotaxin are

elevated in patients with atopic dermatitis.(454) It has been found in the colonic mucosa of patients with both CD and UC with higher levels of expression in inflamed CD mucosa.(441)

IL-21 is a pleiotropic cytokine that acts via the JAK-STAT signalling pathway by binding to IL-21R. It is produced by the Natural Killer T Cells, Follicular T cells, and TH17 cells. IL-21R is activated by binding to a gamma subunit shared with IL-2, IL-4, IL-7, IL-9, and IL-15 and is a key modulator in TNF- $\beta$  signalling. TNF- $\beta$  production is important in the differentiation of T regs and TH17 cells. Higher levels of IL-21 have been associated with greater levels of disease activity in rheumatoid arthritis, SLE, and psoriasis. Genome wide association studies have identified an association with either the IL-2–IL21 genetic locus or the IL-21R with, psoriasis, multiple sclerosis, coeliac disease, UC and CD.(437) In both CD and UC increased expression of IL-21 and IL-21R have been noted. Elevated levels of these proteins have been reported in the mucosal biopsies of patients with CD and UC patients compared with controls.(455) In mouse models of colitis, increased IL-21 is found in mucosal tissues and IL-21 knock out mutation ameliorates the inflammation and lowers cellular infiltration.(71) IL-21 treated cell cultures have increased synthesis of the Macrophage Induced Protein(MIP)-3 $\alpha$  chemokine and the cell culture supernatants supernatant has also been shown to enhance lymphocyte migration in vitro.(455) This suggests that IL-21, either directly or through interaction with TH-17 cells and/or chemokines such as MIP-3  $\alpha$ , is important in lymphocyte migration.

Our data indicate that VDZ exerts a rapid effect on the tissue microenvironment with changes evident within 24 hours. VDZ downregulates expression of cytokines involved in lymphocyte migration in the gut. IL-21, IP-10 and MDC are all known to be involved in lymphocyte migration. Thus, that this might be one pathway that VDZ exerts its anti-inflammatory effect is biologically plausible.

Incubation of patient derived explant culture medium with PBMCs was designed as an experimental method to assess the interaction between the local tissue microenvironment with cells of the systemic immune system. In our study we assessed whether the reported changes in serum

concentration of eotaxin, eotaxin-3, and IL-15 during VDZ induction (see **Chapter 3**) might result from the interaction of the VDZ altered local tissue microenvironment, as represented by VDZ treated UC explant conditioned media with cells of the peripheral immune system i.e., the PBMCs. In effect we looked for evidence of cross talk between the UC explant secretome and the immune cells

In this study, stimulation of PBMCs by VDZ treated UC explant conditioned media did not result in detectable secretion of eotaxin or eotaxin-3. However, IL-15 secretion by PBMCs was significantly reduced following exposure to VDZ treated UC explant conditioned media compared to the IgG treated controls.

High levels of eotaxin were found in serum(419) and colonic biopsies in UC and CD when compared to controls.(421) However, undetectable levels of eotaxin in PBMC culture supernatant has also been reported by others.(456) In eotaxin expression studies, epithelial cells have been shown to express eotaxin in response to IL-1 $\beta$ , TNF- $\alpha$ , and IFN- $\gamma$ . B cells isolated from patients with CD have been reported to secrete eotaxin in response to LPS.(457) Using immunofluorescent analysis of colonic biopsies in paediatric UC, the expression of eotaxin has been shown to be restricted to epithelial cells and CD68+ macrophages.(422)

IL-15, a pro-inflammatory cytokine, is expressed by monocytes and macrophages and has IL-2 like activity in expanding CD8 T cells and NK cells.(458, 459) However unlike IL-2, it inhibits regulation of T cells by inhibiting IL-2 stimulated activation of induced cell death.(459) Higher concentrations of IL-15 have been reported in the serum and tissue in IBD. Higher concentrations of IL-15 are also expressed by PBMCs isolated from UC patients.(460) However, unexpectedly patients treated with adalimumab have been reported to have higher concentrations of IL-15 when compared to controls.(439)

Our study indicates the increase in serum eotaxin and eotaxin 3, observed in association with VDZ induction, is unlikely to be the result of cross talk between PBMC and gut tissue secretome,

however that such cross talk occurs is evidenced by the lowering of IL-15 secretion by PBMCs stimulated by the VDZ treated UC explant conditioned media. Elevations of serum eotaxin and eotaxin 3 following VDZ induction may reflect other pathways of VDZ action, possibly involving epithelial cells and macrophages.

No significant changes eotaxin or eotaxin-3 concentrations in UC explant conditioned media were seen in the short time frame of our study. However, there may be a significant lag time between serum changes and detectable alterations within the tissue microenvironment. This may in part contribute to the delay in VDZ response which can take up to 12 weeks to reach its full effect.

IL-15 concentrations decreased in serum and in PBMC secretion following VDZ exposure. As IL-15 promotes cytokeratin rearrangement and is also involved in lymphocyte migration and extravasation, the reduction in IL-15 in response to VDZ may contribute to the therapeutic action of VDZ. Higher levels of IL-15 have been reported in adalimumab recipients, however in our study IL-15 concentrations were decreased both in the serum following VDZ induction (**Chapter 3**) and from PBMCs following stimulation with VDZ treated explant culture medium. This reduction in IL-15 may be VDZ specific and potentially an important component of VDZ action in modulating the inflammatory response which will require further investigations.

This study confirms that the explant culture media technique is a helpful adjunct in the characterisation of the UC inflammatory tissue microenvironment and will be useful in facilitating further study of the inflammatory proteins in IBD.

These data suggests that, in addition to VDZ's primary action abrogating immune cell trafficking to the gastrointestinal tract, VDZ may have secondary local effects on the colonic microenvironment in UC involving pathways independent of the  $\alpha_4\beta_7$  Integrin.

Further studies to better understand the effect of anti-TNF therapy and the mechanism of action of VDZ are required. Using these techniques in studies with larger patient numbers is warranted. Further explant studies to explore the reasons for anti-TNF failure with greater granularity

regarding the duration of therapy, timing of and nature of failure could be beneficial. Obtaining colonic samples from patients established on VDZ therapy and comparing with those of patients established on other biologic therapies and with controls would be a useful next step. A prospective study of patients commencing VDZ with repeat biopsy at a key time point e.g 14 weeks and compare the cytokine response in explant culture media over time within each patient among responders and non-responders could be more informative in profiling responders and identifying early markers of disease failure.

Examination of the effect of VDZ treated explant culture on PBMCs isolated from the patient providing the explant biopsy could give better insight into the complex interactions between the tissue micro-environment and the systemic immune system of patients with IBD and reduce confounding factors caused by using a healthy control.

#### 4.7 Conclusion

These studies demonstrate how patient derived UC explant culture media can be used in the investigation of the inflammatory protein secretion in the colonic microenvironment in IBD and how therapeutic agents might modulate the inflammatory process.

In our study prior anti-TNF had no evident enduring effect on the 35 inflammatory proteins analysed, however with a larger cohort some of the smaller numeric differences noted might attain significance. There may be other factors, not tested here, that are altered following anti-TNF therapy and contribute to the known association of prior anti-TNF treatment with higher VDZ failure rates.

As evidenced by the results of analysis of the UC explant secretome, VDZ incubation exerts a measurable effect on the gut tissue microenvironment within 24. In this study the concentrations of IP-10, MDC and IL-21 were all reduced following VDZ treatment of UC explants when compared to controls. Reduction in these three inflammatory proteins could contribute to VDZ's anti-inflammatory effect in IBD. While the differences in the concentrations did not meet the threshold

of significance following correction for multiplicity, this early indication of effect warrants further research and confirmation in an expanded.

PBMC expression of IL-15 was altered following incubation with VDZ treated explant conditioned media, indicating that there may be some cross talk between the secretome of VDZ treated UC explants and peripheral immune cells.

## Chapter 5.0: Discussion

### 5.1: Aims of the Thesis

This thesis evaluates potential predictive markers of vedolizumab (VDZ) treatment outcome in patients with inflammatory Bowel Disease (IBD) to enable early identification of those patients most likely to benefit from VDZ and those at risk of failing treatment.

A number of studies are included:

1. Evaluation of the clinical utility of trough VDZ concentrations in patients established on VDZ; the effect of prior anti-TNF exposure or concomitant medications (azathioprine, methotrexate) on maintenance trough concentrations and the interaction of maintenance trough concentrations with serum CRP, serum albumin, and faecal calprotectin.
2. Examination of VDZ trough concentrations during induction as predictors of treatment response and the interactions of VDZ with the known biomarkers (CRP, serum albumin and faecal calprotectin) of disease activity.
3. Characterisation of the inflammatory profile (serum cytokines, chemokines, and growth factors) in the serum of patients with IBD at VDZ initiation and the effect of VDZ induction on that profile to identify a biomarker predictive of treatment outcome
5. Characterisation of the inflammatory profile in the colonic tissue microenvironment using UC-explants, the effect of prior anti-TNF therapy and VDZ on the explant secretome and the interaction of the secretome with PBMCs

### 5.2: Summary of Thesis Findings

IBD is a chronic progressive disease of the digestive tract characterised by periods of clinical relapse and remission for which no definitive cure has been found. The incidence and prevalence of IBD is increasing in the Western world. There is a bimodal age distribution with peaks in the second to fourth decade and again a smaller peak in the sixth and seventh decades of life, however IBD can present at any age, from the very young to the very elderly.

The immunology of IBD is complex and involves dysregulation of the immune system with loss of the barrier function of the gut mucosa. The exact cause remains elusive but involves a combination of environmental, genetic and microbiotic factors. Unravelling the immune pathogenesis has identified targets for therapeutic intervention: e.g., recognition of the importance of the  $\alpha_4\beta_7$  integrin in gut lymphocyte migration identified this as an important therapeutic target culminating in the development of VDZ.

The first accepted description of IBD appears to have been in the late 1700's. After some hazardous false starts the first effective treatments were not developed until the middles of the twentieth century. Treatment options have expanded and are greatly improved since then. There are currently more than 15 different therapeutic options available and more than 10 new agents developed in the last 20 years. Important recent advances have included the development of biologic therapies that have rapidly become a mainstay of maintenance treatment but have not yet displaced steroids as a key component in the management of acute severe flares of IBD.

There is currently no available curative treatment other than surgery for UC, and that can be associated with significant complications that require lifelong treatment. Despite the wealth of therapeutic options, many patients fail to respond to therapy with more than fifty percent of patients requiring a change in therapy within five years. Yet, there is a subset of patients who respond early and have a durable response with long term remission and without need for change in therapy. As IBD is such a heterogenous condition, not every treatment works for all patients. Individualisation of treatment based on biomarkers of response aiming for a precision medicine approach. This study endeavours to assess whether measurement of serum drug levels or inflammatory proteins in serum or tissue could aid the positioning of VDZ in the treatment of IBD.

**Chapter 1:** Following a recap of the history, clinical presentations, epidemiology, and immunology of IBD the chapter focused on presenting a comprehensive overview of currently available therapies and their development history. The current evidence base for disease monitoring, drug level monitoring, and effectiveness of concomitant therapy is discussed.

Although much excellent research has focused on the immunology and the determinants of treatment success or failure the identification of a biomarker predictive of outcome remains elusive. An ideal biomarker is non or minimally invasive, sensitive and disease specific, cost effective, and readily available.(461) Oncostatin M has shown potential as a marker of treatment response to IFX. Currently there is no agreed biomarker predictive of VDZ treatment response.

**Chapter 2:** I investigated the clinical utility of two strategies of VDZ monitoring; a trough level taken during a routinely scheduled clinic visit at any timepoint during maintenance therapy and a trough level scheduled for week 6 of induction. The research questions were whether VDZ trough levels are clinically relevant for patients established on therapy and whether trough drug levels taken at a fixed time point during induction could inform patient management. Trough levels obtained from patients established on maintenance therapy did not correlate with disease activity scores. There was no correlation with accepted markers of disease activity; serum CRP, serum albumin or faecal calprotectin. Importantly, given the requirement for some patients to receive concomitant therapy, neither azathioprine nor methotrexate affected VDZ trough levels.

This work identified week 6 as an important time point in the treatment course. Higher week 6 VDZ trough concentrations associated with week 14 and week 30 steroid free remission and treatment persistence.

Although assessing the benefit of dose optimisation was beyond the scope of this project and is better addressed within a randomised clinical trial, the week 6 drug level has clinical utility in identifying patients more likely to fail induction and who could be suitable for dose optimisation if such proves effective. Using ROC statistical analysis we identified a week 6 trough VDZ of 15.5µg/mL or higher as associated with steroid free remission at week 14 and week 30. Consistent with this result, in a separate analysis, those with a week 6 trough concentration of  $\geq 15.5\mu\text{g/mL}$  were also more likely to persist on treatment compared with those with a trough level  $< 15.5\mu\text{g/mL}$  ( $p=0.004$ ).

Obtaining the trough VDZ concentration at week 6 is of practical clinical use to identify patients at higher risk of treatment failure and who might benefit from closer clinical monitoring.

Findings in the study included that patients with higher baseline CRP are more likely to fail therapy and that at week 6 there is a moderately strong negative linear association between CRP and VDZ. In situations where VDZ drug levels are not readily available, a low CRP or a declining trend in CRP can provide a level of reassurance regarding the adequacy of VDZ therapy.

**Chapter 3:** Here we identified several proteins involved in regulation of inflammation that warrant further investigation. Higher baseline serum concentrations of TNF- $\beta$  and lower concentrations of IL-22, VEGF-C, and IL-7, and were associated with week 14 SFR, while higher IL-4, MDC, and MCP4 and lower IL-10 were associated with week 30 SFR. Based on what is already know regarding the actions of these inflammatory proteins, these associations have biologic plausibility. The fact that no marker reached the threshold of significance following multiple test correction and that there is a lack of consistency in results pertaining to week 14 and week 30 steroid free remission may relate the inherent limitation of the study given the number of study participants. More likely it is reflective of the complexity of the interactions between the host, the disease condition, and triggers for disease flares. As yet, the real world practical clinical application of these as biomarkers of clinical response is limited. However, these findings represent a significant starting point upon which future work can build.

In univariate analysis, baseline concentrations of eight inflammatory proteins associated with steroid free remission; lower concentrations of IL-7 and IL-22, VEGF, and IL-10 and higher concentrations of TNF- $\beta$ , MCP-4, MDC associated with either week 14 or week 30 SFR. Lower baseline concentration of IL-7 was also significantly associated with adequate maintenance VDZ trough concentrations. As the only biomarker predictive of adequate VDZ trough concentrations, this is a potentially important finding with clinical applicability. IL-7 is a potent immunostimulatory cytokine, overexpression of which is associated development of colitis mouse model.(398) It has been proposed as an important element contributing to treatment-refractory, relapsing, or

chronically active disease.(399) Our data is consistent with this in showing the correlation of higher levels of IL-7 with persistent disease. Thus high baseline levels of IL-7 could be used to identify patients who would benefit from closer clinical monitoring and who could be suitable for early dose escalation to ensure adequate VDZ trough levels.

Eotaxins are a group of chemoattractants constitutively expressed in gastrointestinal tract as well as in the thymus and in skin.(170) Significant increases in eotaxin and eotaxin-3 concentrations were documented following VDZ induction. This finding was robust and withstood statistical correction for multiplicity. This is the first time that eotaxin concentrations have been shown to be affected by VDZ. Both eotaxin and eotaxin-3 are pro inflammatory cytokines and thus an increase in their concentration following VDZ is unexpected. There remain unanswered questions regarding their role in IBD.

Although in this study we did not find an association between baseline IL-15 level and disease status at week 14 or 30 there was a significant decline in the concentration of IL-15 following VDZ induction. IL-15 is a pro inflammatory cytokine that is important for movement and extravasation of lymphocytes. As the VDZ acts through inhibition of lymphocyte migration, primarily thought due to blocking the  $\alpha_4\beta_7$  integrin, finding that VDZ also leads to a reduction of IL-15 suggests that this may represent an additional pathway through which inhibition of lymphocyte migration is achieved.

**Chapter 4:** The UC explant model was adapted and used to determine the effect of VDZ on secretome of colonic tissue to reflect the *in-vivo* changes of the tissue microenvironment. Following a 24-hour incubation with VDZ, alterations were seen in the secretome of incubated explant. This indicates that VDZ rapidly exerts a direct effect on local tissue. Concentrations of IP-10, MDC and IL-21 were lower in the VDZ treated samples compared to controls. Although these finding did not meet the threshold of significance following multiple test correction, that the findings are clinically significant is biologically plausible. These three cytokines are pro inflammatory and are associated with lymphocyte migration. Finding that VDZ reduces their concentrations is consistent the known effects of VDZ on lymphocyte migration.

None of the serum proteins identified in **Chapter 3** as associated with either steroid free remission, or that had significant change in their concentration following VDZ induction, were found to undergo similar changes in the explant model. This discordance could represent a limitation of the study given the numbers tested, or that there is a lag times between the effects detectable in serum and at the tissues level, or that the discordance indicates that there are different pathways of VDZ's activation depending on the environment.

Vedolizumab's primary mechanism of action is as an  $\alpha_4\beta_7$  integrin antagonist inhibiting lymphocyte migration. Were this the only mechanism of action a minimal effect on the local tissue microenvironment might be expected. However, it has been proposed that VDZ's mechanism of action extends beyond inhibition of lymphocyte migration. It has been shown to affect macrophage populations, leading to down regulation of macrophage associated genes. Although our work can neither confirm nor exclude that VDZ's actions extend beyond inhibition of lymphocyte migration the rapidity of its' effect on the local tissue microenvironment suggest that there could be a more direct action that may be independent of  $\alpha_4\beta_7$  integrin.

The findings in this chapter, while not definitive pave the way for further research on inflammatory proteins in IBD and mechanisms of VDZ's action at the local level.

### 5.3: Study Applications

This study adds to the existing knowledge regarding IBD and specifically to the evidence base required to optimally manage patients receiving VDZ. While evidence to support clinical utility of routine VDZ trough levels during maintenance therapy was not found, pre-infusion sampling at week 6 of induction was identified as an important time point in the treatment course.

Week 6 trough levels were predictive of week 14 and week 30 steroid free remission and of treatment persistence. Thus, a trough level could potentially be added to the routine week 6 clinical

assessment to identify those patients likely to successfully continue VDZ and perhaps more importantly identify those at increased risk of failure so that closer monitoring might be initiated.

In the situation where trough VDZ levels might not be available, the finding that a normal CRP (<5mg/L) was predictive of steroid free remission and that CRP during induction associated with VDZ trough levels, albeit with some outliers, suggest that it might be used as a surrogate for VDZ trough levels in determining the frequency of monitoring required.

In this thesis, a number of inflammatory proteins that warrant further investigations are identified. The results should be regarded as preliminary and warrant confirmation. However, the findings are biologically plausible based on what is already known regarding these proteins. Ultimately given the current state of knowledge the immediate clinical applicability of these findings in the management of patients undergoing VDZ treatment is limited. However, the research undertaken here permits narrowing of the focus of future research to identify a biomarker or biomarker profile predictive of treatment success.

As profiling inflammatory markers in the serum does not necessarily reflect that occurring at the tissue level, a model that allows exploration of drug interactions in the tissue micro-environment could facilitate development of therapeutic agents for IBD, lead towards a precision medicines paradigm, and aid the development of novel therapeutic agents. The work presented confirms the feasibility and potential value of the UC-explant model in the investigation of novel IBD agents. This explant model may prove a useful tool in progressing the understanding IBD pathogenesis and rationalise use of available therapeutics.

The identification of the ideal biomarker of treatment response remains elusive. However, the findings reported in this thesis pave the way for further research on the identified inflammatory proteins both in serum and, with the UC-explant model, in the tissue microenvironment.

#### 5.4: Overall Strengths and Limitations of the Thesis

This thesis incorporates a number of studies that collectively focus on the VDZ and the search for biomarkers of treatment response.

In a logical progression, I focused on the clinical relevance of serum drug levels during maintenance and during induction therapy, the impact of VDZ of inflammatory proteins in the serum, the effect of VDZ on inflammatory proteins in the colonic tissues microenvironment and the potential for cross talk between the gut tissue secretome and cells of the peripheral immune system.

As all study centres were university affiliated academic hospitals and IBD tertiary referral centres the cohort, recruited over a 19 month period, was well characterised and relatively large. Retention on study was excellent. In the prospective induction study the retention was 90% retention rate at the week 30 endpoint and VDZ persistence data was available on 97% patients at two years.

A particular strength of the maintenance and induction studies were their pragmatic nature. Decisions regarding VDZ administration, dosing interval, and concomitant drug use left to the discretion of the treating physician reflecting the real-world experience of VDZ in clinical practice. These studies highlight the ability to incorporate scientific research into standard clinical care and that the approach is acceptable to the patient population as was evidenced by the low default rate.

The importance not only of drug pharmacokinetics but also the pharmacokinetic-pharmacodynamic interactions is recognised in the overall investigation plan. Following the focus on trough drug levels (**Chapter 2**), the interactions of VDZ with biomarkers of disease activity (serum CRP, albumin, and faecal calprotectin) (**Chapter 3**) and VDZ effect on serum concentrations of a broad range proteins involved in the inflammatory process (**Chapter 3**) (54 different cytokines, chemokines and growth factors) were researched.

Bringing the investigation even closer to the source pathology, a series of investigations were undertaken researching the impact of VDZ within the colonic tissue microenvironment. To do this an explant model of proven utility in cancer drug development was adapted and used. In doing this not only has the study added to the knowledge base pertaining to VDZ but demonstrates the

feasibility of using the explant model to investigate inflammatory proteins in the gut. This will have application in the development of other novel agents IBD.

The assays involved were performed under supervision in a laboratory with an established track record in IBD research and with collaborative engagement with researchers experienced in the use of explants in cancer drug development.(401)

A significant strength of the studies was the duration of follow up that extended up to 41 months and the completeness of the data sets, the collection of which was co-ordinated and managed by the author.

That the study was carried out in three academic referral centres opens the possibility of the inherent referral centre bias with a weighting towards patients that may be less representative of the general IBD population and with greater complexity. However while the centres are national referral centres, they also provide secondary level care to the respective local catchment areas, thus patients attending encompass the full spectrum of IBD.

Although validated, well characterised disease activity scores with long history of use were employed as primary endpoints in the VDZ induction study, endpoints did not incorporate histologic or endoscopic remission. Current endpoint guidelines from both the EMA and FDA for use in clinical trials for IBD recommend endpoints including both symptomatic and endoscopic remission.(462) Endoscopic outcomes were not obtained, due to the real world pressure on endoscopy slots. Unfortunately, although it is desirable for all patients with IBD to undergo endoscopy after six months of treatment it is not always feasible.

In the VDZ induction study treatment persistence for up three and a half years, an accepted hard endpoint of treatment success, was assessed. In it (**Chapter 2**) and in the study of VDZ and serum inflammatory proteins (**Chapter 3**) disease activity index scores, such as the partial Mayo score or a Harvey Bradshaw index score were used to determine remission status. There has also been a recent shift in emphasis from using specifically defined medical targets, such as disease scores, and

endoscopic data to placing greater significant on patient reported outcomes. A more holistic approach incorporating drug acceptability and quality of life questionnaires could bring greater depth to our understanding of the clinical utility of this drug in treating IBD.

The numbers of patients recruited into the explant study although numerically small are relatively large for this type of study. None the less, small numbers increase the risk of introducing type II error in interpreting the results which may be further compounded by the stringency of the false discovery rate control used. This introduces the risk of failure to recognise the clinical significance of the results. Given the incidence and prevalence of IBD within the community, collaborative engagement with wider research networks could increase the number of participants, strengthen the results and add to the generalisability of the findings.

A limitation of the study is that the *ex-vivo* explants were treated *in vitro* with VDZ. While it is assumed that it is reflective of the gut tissue microenvironment, future studies using UC explants derived from patients on VDZ treatment could confirm this assumption.

The cellular composition of the colonic explant biopsies was not investigated in this study. Analysis of the cellular composition to determine the level of lymphocyte infiltration and assess the other cellular components could help clarify some of the variability in response to VDZ.

A possible limitation is that the PBMCs used in the exploration of cross-talk with the UC explant secretome were collected from a healthy donor and may not be representative of those from a patient with active IBD. Use of PBMCs isolated from the patient providing the explant could give a more accurate reflection of the PBMC-secretome cross talk that occurs *in vivo*.

Overall this thesis provides a detailed body of work pertaining to VDZ in the treatment of IBD in a well characterised population, adds to the current literature on IBD research and opens avenues for further research.

## 5.5: Areas of Future Research

This thesis has expanded upon our knowledge of the clinical use of VDZ. A state of equipoise exists regarding the merits of VDZ drug monitoring and the clinical relevance of VDZ levels. Randomised controlled trials are needed to provide a definitive answer and inform clinical. Our data suggests that a week six assessment could provide clinically relevant information, however this requires confirmation. Low drug levels at week six of induction are associated with disease persistence.

Our data is consistent with that of others indicating that VDZ is not very immunogenic and is associated with low rates of anti-drug antibody formation(313, 314). Drug inactivation consequent to antibody neutralisation is unlikely to account for the low and possibly sub-therapeutic levels found in patients failing therapy. Low VDZ levels could reflect diseases severity rather than inadequate drug dosing. Low levels may indicate more rapid drug metabolism and. To date the evidence supporting dose intensification is weak however it has not yet been tested in the setting of a randomised controlled trial. Given the patients numbers required, this might only successfully happen in the context of a large multicentre trial comparing, proactive (scheduled) dose monitoring and optimisation, with reactive dose monitoring and optimisation (based on clinical status) and standard eight weekly dosing of VDZ.

In treatment with IFX the use of a 'dashboard', that calculates the optimal induction dose based on biochemical and clinical factors has been trialled and has been shown to increase treatment durability and reduce immunogenicity.(231, 463) In our study, serum albumin and CRP correlate with VDZ concentration during induction. The development of a similar dashboard for VDZ dosing and trialling the effect of this on VDZ induction could be of benefit.

In the analysis of the serum inflammatory profile of patients receiving VDZ induction, we identified several cytokines associated with treatment failure based on disease activity scores. Given the stringency of the multiple test correction none reached the threshold of significance however that does not necessarily exclude that they might be clinically significant. By undertaking a similar study with a larger cohort a more definitive answer regarding the clinical relevance could be achieved if

in addition such a study to incorporate as endpoints histologic and endoscopic remission at week 14 and week 30.

The effect of VDZ induction on the concentrations of eotaxin, eotaxin-3 warrants further investigation as these were unexpectedly increased following VDZ induction. As eotaxin and eotaxin 3 primarily act as chemo-attractants for eosinophils, evaluation of the cellular composition of colonic biopsies specifically looking for eosinophils in VDZ treated patients would be of interest and could expand therapeutic options.

We have shown the feasibility of using the UC explant model to investigate the secretome of colonic tissue. The model could be used to investigate the actions of novel agents on the secretome and to extend our findings on the cross talk that occurs between colonic cells and cells of the systemic immune system. Based on the findings in this thesis a study with larger patient numbers and with a more focused approach concentrating on the cytokines and chemokines identified as of interest could be successful in identifying biomarkers of clinical response

Our studies have confirmed the feasibility of using colonic explants to study the colonic secretome and the effect that an agent such as VDZ can have on it. It would be of interest to incorporate explants from remitting and non-remitting patients and at different times throughout their treatment course into such studies. Colonic biopsies obtained at initiation of VDZ and subsequently at set time points e.g., week 6 and week 30 or at time of clinical failure, could build on this work, furthering the investigation of the explant secretome to identify potential biomarkers of disease outcome.

Examination of the secretome of explants from UC patients undergoing VDZ induction is a next step. Comparing explants from patients undergoing colonoscopy before VDZ induction with explants obtained at week 30 would be of interest as VDZ can take up to 12 weeks exert its full effect. Comparing the changes with patients undergoing IFX induction used as a control could help identify VDZ specific changes in the secretome.

The explants in this study were derived from patients with ulcerative colitis. While there are many similarities between CD and UC, there is much that is unique to each condition. A similar explant study investigating the effect of VDZ incubation on either colonic explants or terminal ileal explants derived from patients with CD and comparing results of the secretome between UC colitis and Crohn's colitis and between intestinal and colonic biopsies could help the understanding of some of the differential responses seen in patients with UC and CD to therapeutic agents.

## 5.6: Conclusions

These translational studies support the effectiveness of VDZ for treatment of IBD. Findings in this thesis add to the existing knowledge base regarding VDZ in IBD and its effects on both immune responses and clinical endpoints.

Week 6 VDZ trough levels proved effective in predicting patients at risk for treatment failure, a finding with real world clinical utility. Baseline CRP was shown to have clinical utility in identifying patients at risk of disease failure. Serum CRP and albumin at the time of the third and fourth infusion correlate well with VDZ trough levels and could potentially be used as a surrogate marker to indicate adequacy of VDZ dosing. CRP is a widely available cheap clinical tool that clinicians are extremely familiar with.

Although no serum biomarker of treatment response was definitively identified, several potential targets for further investigation were noted and may guide future research. VDZ induction was shown to have significant effect on the serum inflammatory profile of patients and to have a rapid onset of action within the local tissue microenvironment that could possibly be independent of the  $\alpha_4\beta_7$  integrin pathway.

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