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SUPPLEMENTARY METHODS

Data collected

A pro-forma detailing the inclusion and exclusion criteria, baseline demographics, and necessary data for collection was sent electronically to participating centres, and data was collated centrally (by KDL). Efforts were made to collect missing data by electronic correspondence with participating centres. Patients for whom data were incomplete in respect of treatment or alkaline phosphatase (ALP) at baseline and follow-up were excluded from the analysis. Endoscopic data was provided where available; however the lack of endoscopic scoring for IBD prior to the start of vedolizumab and at last follow up was not regarded as an exclusion criterion.

Baseline demographics collected included gender, age at diagnosis of PSC, type of PSC, presence of cirrhosis, type of IBD, race and ethnicity. The presence or not of cirrhosis was determined by the treating clinician according to clinical parameters, imaging and liver biopsy (where available). Whether vedolizumab had been discontinued was recorded, as was the reason for discontinuation (if known), the duration of vedolizumab treatment, baseline ursodeoxycholic acid (UDCA) use and dose, and past use of anti-tumour necrosis factor (TNF) medication.

Clinical laboratory parameters collected at baseline and follow-up included serum ALP, alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin and albumin, platelet count and international normalised ratio (INR). ALP was measured in international units per litre (IU/L) and calculated times the upper limit of normal (xULN). Follow-up laboratory tests were included up until liver transplantation (LT), death, or 56 days following the final vedolizumab infusion (as this is the usual duration between maintenance infusions), whichever occurred first.

Time-points of liver biochemistry data

Liver biochemistry data (ALP, ALT, AST, and bilirubin levels) were collected at baseline, week 6 (W6, i.e. day 42), W14 (i.e. day 96) and last follow-up whilst on vedolizumab. The time points of W6 and W14 were used as early and medium time-points to assess change in liver biochemistry as these correspond with the last induction dose of vedolizumab and the first maintenance dose time-points. In clinical practice, the response of IBD to vedolizumab treatment is typically assessed after 6 to 10 weeks.[1, 2]

Liver biochemistry results were included up until 56 days after the final vedolizumab infusion, as 56 days is the interval between vedolizumab maintenance doses. For these time-point analyses, only patient data that had paired baseline and the appropriate time-point data were used. To allow for variation in clinical practice, laboratory test results from days 30-59 could be assigned as the W6 value, and from days 90-119 were used for the W14 value.

Statistical analyses

In comparing continuous variables from baseline to a later time point, paired t-tests or Wilcoxon matched-pairs signed rank tests were used according to the whether the data was distributed parametrically or non-parametrically respectively.

Univariate logistic regression was carried out for the outcomes of ALP reduction ≥20% from baseline to last follow-up, ALP rise ≥20% or more from baseline to last follow up, ALP<1.5xULN at last follow-up (within subgroup with baseline ALP>1.5xULN), the presence of liver-related outcomes, and improved endoscopic IBD response (versus worsened/ unchanged). Multivariate logistic regression was carried out to assess the impact of relevant variables on ALP drop or rise by 20% or more from baseline to last follow-up. Separate univariate and multivariate logistic regression was carried out on ALP drop by 20% or more for the cohort of patients who were not also on UDCA at vedolizumab initiation, as UDCA use can lower ALP. Where there were missing covariates, a complete case analysis was carried out (see *Supplementary Table 2*).

The statistical software used was Graphpad Prism version 7.0a for Mac OS X (GraphPad Software, La Jolla California USA, www.graphpad.com), and for the univariate and multivariate analyses, R 3.5.1 Core Team (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, 2018. URL http://www.R-project.org/.)

SUPPLEMENTARY RESULTS

Duration of vedolizumab and follow-up

The median duration of vedolizumab treatment was 412 days (IQR 180-651, range 37-2609). and patients were followed up with regards to clinical endpoints for a median duration of 561 days (IQR 325-790, range 42-2614). All patients had received a minimum of three infusions at the usual dosing schedule, except for one patient whose third infusion was slightly earlier on day 37 rather than day 42/W6. Forty-four patients (43.1%) had their vedolizumab treatment stopped during the study period. The majority of treatment cessation was due to lack of efficacy (32 patients, 72.3%), with six (13.6%) stopping due to an adverse reaction and another six stopping for unknown reasons.

Timepoints of last follow-up liver biochemistry variables

The median duration post-vedolizumab of the last follow-up laboratory measurement varied depending on the biochemistry variable. These were:

- ALP: 374 days (IQR 167-579)
- ALT 374 days (IQR166-613)
- AST: 438 days (IQR208-641)
- bilirubin: 385 days (IQR 167-637).

Outcomes on subsets of patients

Patients with cirrhosis

Within the cohort of patients with cirrhosis (n=21) no correlation was found between change in ALP and change in other parameters of liver and organ function such as INR, bilirubin and creatinine (data not shown).

Patients not on UDCA

Separate univariate and multivariate analyses were carried out on the subgroup of patients who were not on UDCA at baseline (n=41, see *Supplementary Table 4*). Ten patients (24.4%) had an ALP drop ≥20%; cirrhosis again was associated with this outcome in this cohort on univariate analysis, but not on multivariate analysis.

Patients with baseline ALP >1.5xULN

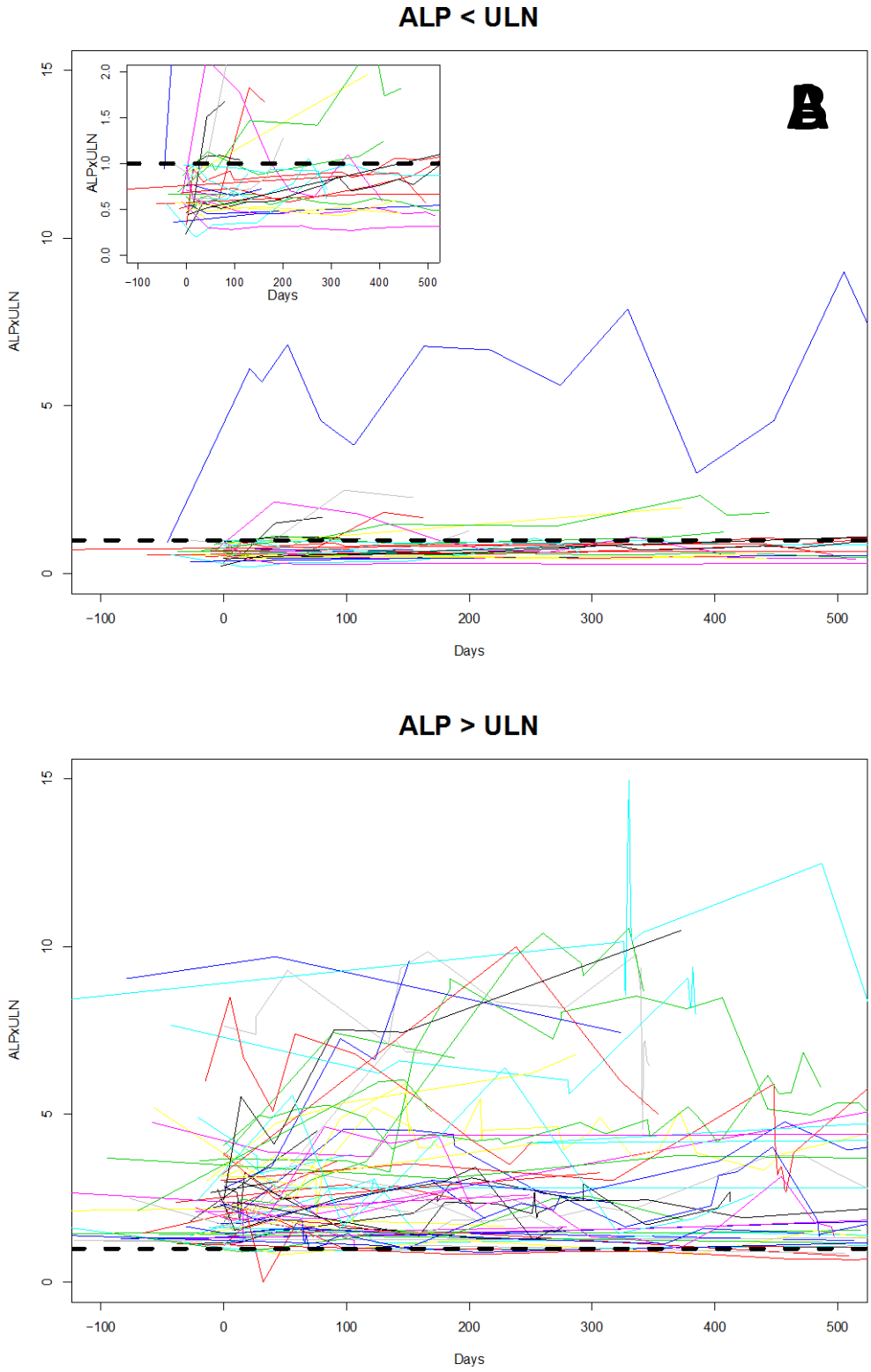
Given the previous evidence for a worse prognosis in patients whose ALP is >1.5x upper limit of normal (ULN),[20-22] we also evaluated within the subgroup of patients whose baseline ALP was >1.5xULN, looking at the outcome of the proportion whose ALP dropped to <1.5xULN at last follow-up.

When analysing this subgroup of patients (ALP>1.5xULN at baseline, n=51) 10 (19.6%) had ALP<1.5xULN at last follow-up. Univariate analyses revealed that only type of IBD was associated with this outcome, with an OR of 0.07 (95% CI 0.013-0.39, p=0.002) for UC as compared with CD/IBD-u (see *Supplementary Table 5*).

Patients with duration of VDZ >6 months

Seventy-three patients had been on vedolizumab for a minimum of 6 months and had ALP data to >6 months. We analysed this subgroup to determine if a longer duration of vedolizumab treatment had any bearing on the results above. In this cohort, the median ALP did not change from baseline (1.65xULN [IQR 0.93-3.01]) to last follow-up (1.63xULN [IQR 1.03-3.56], p=0.284), and a similar proportion of patients had an ALP drop ≥20% (24.7%) and ALP rise ≥20% (37.0%) compared to the overall cohort. Univariate and multivariate analyses were also similar to those of the overall cohort (data not shown).

FIGURES



Supplementary Figure 1: The trajectory of ALP from baseline over time according to baseline ALP below and above ULN (n=102).

The trajectory of ALP from baseline over time according to baseline ALP below and above ULN (n=102). These spaghetti plots showing the ALP at baseline and different time-points on VDZ. Each line represents a unique patient. The dotted black line represents an ALP of 1xULN. (A) Patients whose baseline ALP was less than 1xULN; (B) Patients whose baseline ALP was greater than 1xULN. The inset in (A) is a magnified portion of the graph to more easily see the trajectory of those below the ULN. VDZ was started at day 0. Patients whose baseline ALP was <ULN tended to follow a fairly stable course, whereas those with baseline >ULN followed a fairly erratic course. ALP, alkaline phosphatase; ULN, upper limit of normal; VDZ, vedolizumab.

TABLES

Supplementary Table 1: Participating Centres and the number of patients (*n*) from each centre included in the overall cohort (n=102).

Centre	Location	*n*
Oxford University Hospitals	Oxford, UK	15
Friedrich-Alexander-University	Erlangen, Germany	10
University of Alberta	Edmonton, Canada	10
Academic Medical Center	Amsterdam, Netherlands	7
University Hospitals Birmingham	Birmingham, UK	7
University Medical Center Hamburg-Eppendorf	Hamburg, UK	7
University of Miami	Miami, FL, USA	6
Massachusetts General Hospital	Boston, US	6
University of California Davis	Davis, CA, USA	6
Karolinska Instituet	Stockholm, Sweden	6
University of Washington Medical Center	Seattle, USA	4
Yale University School of Medicine	New Haven, CT, USA	4
Erasmus Medical Center	Rotterdam, Netherlands	4
University of Copenhagen	Copenhagen, Denmark	3
Medical University of Vienna	Vienna, Austria	2
California Pacific Medical Center	San Francisco, USA	1
University of Gothenburg	Gothenburg, Sweden	1
Ospedali Riuniti University Hospital	Ancona, Italy	1
University of Padova	Padova, Italy	1
Epatocentro Ticino	Lugano, Switzerland	1

Supplementary Table 2: Number of variables missing within the complete dataset (n=102).

Variable	***n***
Presence of cirrhosis	1
Baseline ALP	0
Type of IBD	0
Gender	0
Age at diagnosis of PSC	0
UCDA use at baseline	0
Type of PSC	0
Duration vedolizumab	0
IBD response	28
Previous anti-TNF use	7
Paired baseline follow-up ALP	0
Paired baseline and follow-up ALT	1
Paired baseline and follow-up AST	34
Paired baseline and follow-up bilirubin	5
Baseline platelets	3
Baseline INR	39
Baseline albumin	9

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; IBD, inflammatory bowel disease; INR, international normalised ratio; n, number; PSC, primary sclerosing cholangitis; TNF, tumour necrosis factor.

Supplementary Table 3: Univariate and multivariate analysis for ALP rise by 20% or more from baseline to last follow up.

Variable	Univariate		Multivariate	
	Odds Ratio (95% CI)	**P-value**	**Odds Ratio (95% CI)**	**P-value**
Cirrhosis	0.20 (0.02-1.60)	0.130	0.163 (0.02-1.44)	0.103
Baseline ALP >ULN[a]	0.47 (0.16-1.35)	0.161	0.78 (0.23-2.62)	0.679
Ulcerative Colitis[b]	2.96 (0.79-11.10)	0.107	2.66 (0.67-10.54)	0.165
Male gender	1.52 (0.49-4.72)	0.466	1.52 (0.45-5.19)	0.502
Age at diagnosis of PSC[c]	1.01 (0.97-1.04)	0.767	1.02 (0.98-1.06)	0.416
UCDA use at baseline	0.54 (0.19-1.53)	0.244	0.56 (0.17-1.91)	0.358
Small duct-PSC[d]	1.71 (0.31-9.30)	0.534	-	
PSC-AIH overlap[d]	n/a[e]	n/a	-	
Duration vedolizumab[f]	1.01 (0.95-1.06)	0.832	-	
IBD improved[g]	0.90 (0.24-3.26)	0.873	-	
Previous anti-TNF use	1.53 (0.45-5.18)	0.492	-	

[a]Baseline indicating last ALP taken before VDZ commenced; [b]versus IBD-unspecified or Crohn's disease; [c]per 1 year increase; [d]versus large duct-PSC; [e]There were too few numbers of events to fit the binomial generalised linear model, and therefore an OR could not be calculated; [f]per one month increase in vedolizumab duration; [g]endoscopic improvement versus unchanged/worsened.

ALP=alkaline phosphatase; AIH=autoimmune hepatitis; IBD=inflammatory bowel disease; n/a, not applicable; OR, odds ratio; PSC=primary sclerosing cholangitis; TNF=tumour necrosis factor; UDCA=ursodeoxycholic acid; ULN=upper limit of normal;

Supplementary Table 4: Univariate and multivariate analysis for ALP drop by 20% or more from baseline to last follow up among patients not on ursodeoxycholic acid (n=41).

Variable	Univariate		Multivariate	
	Odds Ratio (95% CI)	**P-value**	**Odds Ratio**	**P-value**
Cirrhosis	7.80 (1.60-38.11)	0.011	5.72 (0.68-47.79)	0.107
Baseline ALP >ULN[a]	2.83 (0.62-13.04)	0.181	2.12 (0.27-16.34)	0.472
Ulcerative Colitis[b]	0.24 (0.05-1.10)	0.067	0.41 (0.06-2.92)	0.370
Male gender	0.42 (0.10-1.81)	0.245	0.42 (0.06-3.03)	0.392
Age at diagnosis of PSC[c]	1.06 (1.00-1.12)	0.053	1.03 (0.95-1.11)	0.468
Small duct-PSC[d]	1.75 (0.14-21.88)	0.664	-	
PSC-AIH overlap[d]	3.50 (0.20-62.42)	0.394	-	
Duration vedolizumab[e]	1.06 (0.98-1.14)	0.157	-	
IBD improved[f]	0.75 (0.14-4.13)	0.741	-	
Previous anti-TNF use	0.83 (0.19-3.75)	0.812	-	

[a]Baseline indicating last ALP taken before VDZ commenced; [b]versus IBD-unspecified or Crohn's disease; [c]per 1 year increase; [d]versus large duct-PSC; [e]per one month increase in vedolizumab duration; [f]endoscopic improvement versus unchanged/worsened.
ALP=alkaline phosphatase; ULN=upper limit of normal; PSC=primary sclerosing cholangitis; AIH=autoimmune hepatitis; IBD=inflammatory bowel disease; TNF=tumour necrosis factor.

Supplementary Table 5: Univariate Analysis for ALP<1.5xULN at last-follow-up, amongst subgroup of patients for whom baseline ALP>1.5xULN (n=51).[a]

Variable	Univariate	
	Odds Ratio (95% CI)	**P-value**
Cirrhosis	0.62 (0.11-3.38)	0.576
Ulcerative Colitis[b]	0.07 (0.01-0.39)	0.002
Male gender	0.34 0.08-1.43)	0.142
Age at diagnosis of PSC[c]	1.02 (0.97-1.08)	0.434
UCDA use at baseline	2.56 (0.48-13.62)	0.270
Small duct-PSC[d]	n/a[e]	n/a
PSC-AIH overlap[d]	n/a[e]	n/a
Duration vedolizumab[f]	1.04 (0.95-1.13)	0.411
IBD improved[g]	0.92 (0.17-4.86)	0.925
Previous anti-TNF use	0.30 (0.07-1.33)	0.112

[a]Baseline indicating last ALP taken before VDZ commenced; [b]versus IBD-unspecified or Crohn's disease; [c]per 1 year increase; [d]versus large duct-PSC; [e]There were too few numbers of events to fit the binomial generalised linear model, and therefore an OR could not be calculated; [f]per one month increase in vedolizumab duration; [g]endoscopic improvement versus unchanged/worsened.
ALP=alkaline phosphatase; ULN=upper limit of normal; PSC=primary sclerosing cholangitis; AIH=autoimmune hepatitis; IBD=inflammatory bowel disease; TNF=tumour necrosis factor.

Supplementary Table 6: Univariate Analysis for Endoscopic IBD Response (n=74).

Variable	Univariate	
	Odds Ratio (95% CI)	**P-value**
Cirrhosis	0.79 (0.27-2.35)	0.677
Baseline ALP >ULN[a]	0.29 (0.10-0.85)	0.024
Ulcerative Colitis[b]	1.01 (0.39-2.61)	0.979
Male gender	1.12 (0.43-2.90)	0.815
Age at diagnosis of PSC[c]	1.01 (0.97-1.05)	0.635
UCDA use at baseline	2.57 (0.97-6.81)	0.058
Small duct-PSC[d]	1.15 (0.18-7.35)	0.880
PSC-AIH overlap[d]	n/a[e]	n/a
Duration vedolizumab[f]	1.08 (1.02-1.14)	0.013
Previous anti-TNF use	1.47 (0.53-4.08)	0.456

[a]Baseline indicating last ALP taken before VDZ commenced; [b]versus IBD-unspecified or Crohn's disease; [c]per 1 year increase; [d]versus large duct-PSC; [e]There were too few numbers of events to fit the binomial generalised linear model, and therefore an OR could not be calculated [f]per one month increase in vedolizumab duration.
ALP=alkaline phosphatase; ULN=upper limit of normal; PSC=primary sclerosing cholangitis; UDCA=ursodeoxycholic acid; AIH=autoimmune hepatitis; IBD=inflammatory bowel disease; TNF=tumour necrosis factor.

Supplementary Table 7: Univariate analysis for liver-related outcome (n=102)*

Variable	Odds Ratio	P-value
Cirrhosis	5.70 (1.96-16.57)	0.001
Baseline ALP >ULN[a]	1.67 (1.23-2.28)	0.001
Ulcerative Colitis[b]	1.12 (0.40-3.08)	0.833
Male gender	1.63 (0.57-4.65)	0.358
Age at diagnosis of PSC[c]	0.99 (0.96-1.03)	0.696
Baseline serum albumin[a,d]	0.90 (0.83-0.98)	0.024
Baseline Platelets[a,e]	0.96 (0.92-1.00)	0.051
Baseline INR[a,f]	1.28 (0.84-1.95)	0.251

*Liver-related outcome defined as any of the following: listing for LT, undergoing LT, ascending cholangitis, new onset ascites, variceal bleed, cholangiocarcinoma, and death. [a]Baseline indicating last lab value taken before VDZ commenced; [b]versus IBD-unspecified or Crohn's disease; [c]per 1 year increase; [d]per 1mg/L increase; [e]per 10 x 10^9/L increase; [f]per 0.1 units increase.
ALP=alkaline phosphatase; ULN=upper limit of normal; PSC=primary sclerosing cholangitis; AIH=autoimmune hepatitis; IBD=inflammatory bowel disease; INR=international normalised ratio; LT=liver transplantation.

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