Manuscript Doi: 10.1093/ecco-jcc/jjae076

# Cyclophosphamide-free mobilisation increases safety while preserving the efficacy of autologous haematopoietic stem cell transplantation in refractory Crohn's disease patients

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Manuscript Doi: 10.1093/ecco-jcc/jjae076

**Non-standard abbreviations**

| | |
|---|---|
| AE | Adverse event |
| AHSCT | Autologous haematopoietic stem cell transplantation |
| ATG | Anti-thymocyte globulin |
| EBMT | European Society for Blood and Marrow Transplantation |
| CD | Crohn's disease |
| CDAI | Crohn's Disease Activity Index |
| Cy | Cyclophosphamide |
| CMV | cytomegalovirus |
| DMSO | Dimethyl sulfoxide |
| EBV | Epstein-Barr virus |
| FDR | False discovery rate |
| G-CSF | Granulocyte colony-stimulating factor |
| HIV | Human immunodeficiency virus |
| HSC | Haematopoietic stem cells |
| HSV | Herpes simplex virus |
| IQR | Interquartile range |
| MRE | Magnetic Resonance enterography |
| OCATT | Organització Catalana de Transplantaments |
| PBMC | Peripheral blood mononuclear cells |
| RT-PCR | Real Time Polymerase Chain Reaction |
| SAE | Serious adverse event |
| SES-CD | Simple Endoscopic Score for Crohn's disease |
| TNF | Tumor necrosis factor |
| VZV | Varicella-zoster virus |

Manuscript Doi: 10.1093/ecco-jcc/jjae076

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Manuscript Doi: 10.1093/ecco-jcc/jjae076

## ABSTRACT

**Background and aim:** Autologous haematopoietic stem cell transplantation [AHSCT] is a therapeutic option for refractory Crohn's disease [CD]. However, high adverse event rates related to chemotherapy toxicity and immunosuppression limit its applicability. This study aims to evaluate AHSCT's safety and efficacy using a cyclophosphamide (Cy)-free mobilisation regimen.

**Methods:** A prospective observational study included 14 refractory CD patients undergoing AHSCT between June 2017 and October 2022. The protocol involved outpatient mobilisation with G-CSF 12-16 μg/kg/daily for 5 days, and optional Plerixafor 240 μg/d (1-2 doses) if the CD34+ cell count target was unmet. Standard conditioning with Cy and anti-thymocyte globulin was administered. Clinical, endoscopic, and radiological assessments were conducted at baseline and during follow-up.

**Results:** All patients achieved successful outpatient mobilisation (7 patients needed Plerixafor) and underwent transplantation. Median follow-up was 106 weeks (IQR 52-348). No mobilisation-related serious adverse events (SAEs) or CD worsening occurred. Clinical and endoscopic remission rates were 71% and 41.7% at 26 weeks, 64% and 25% at 52 weeks, and 71% and 16.7% at the last follow-up. The percentage of patients who restarted CD therapy for clinical relapse and/or endoscopic/radiological activity was 14% at 26 weeks, 57% at 52 weeks, and 86% at the last follow-up. Peripheral blood cell populations and antibody levels post-AHSCT were comparable to Cy-based mobilisation.

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**Conclusions**: Cy-free mobilisation is safe and feasible in refractory CD patients undergoing AHSCT. Although relapse occurs in a significant proportion of patients, clinical and endoscopic responses are achieved upon CD-specific therapy reintroduction.

**Keywords**

Transplant, Crohn, safety

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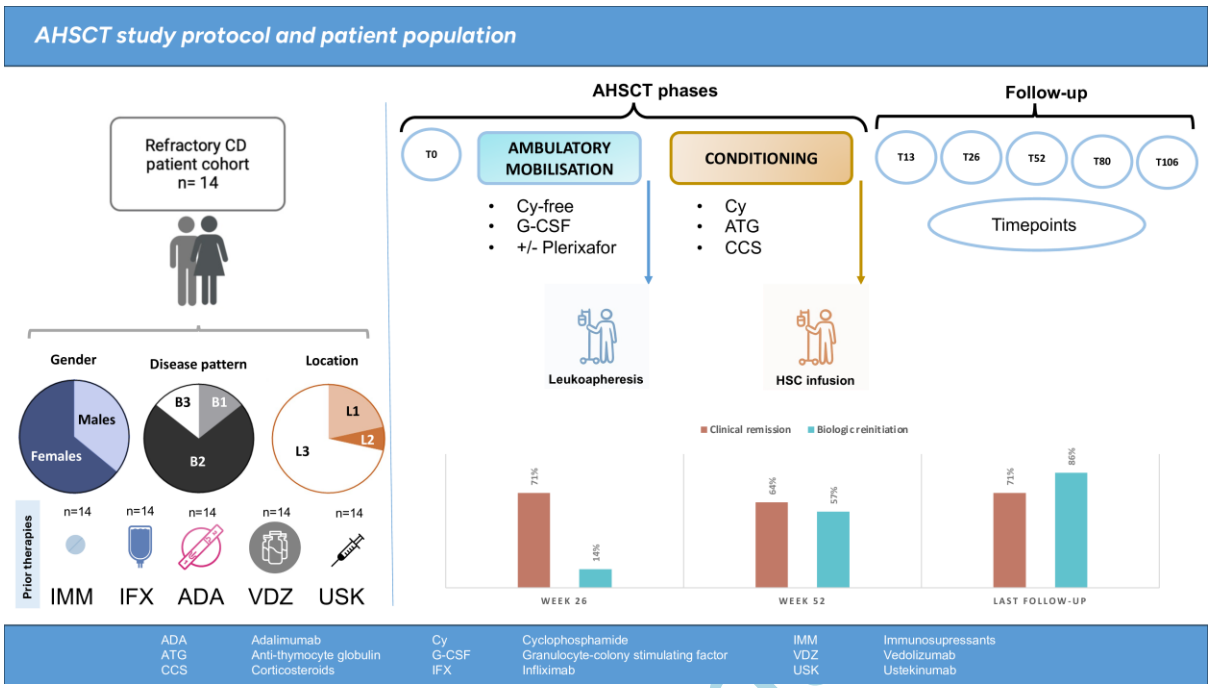
Graphical abstract



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## INTRODUCTION

Therapies for Crohn's disease (CD) have notably evolved in recent decades; however, 30% of CD patients remain refractory to, or lose response to, current treatments.[1] Refractory CD was recently characterized as a condition associated with patients who do not achieve clinical response to all available medical therapies and who are not considered amenable to intestinal surgery.[2] In such cases, the use of autologous haematopoietic stem cell transplantation (AHSCT) is considered a valid therapeutic option.[3]

Case series and one clinical trial (ASTIC) have demonstrated that AHSCT reduces the use of immunosuppressive therapy and increases the rate of clinical and endoscopic remission in refractory CD.[4,5] The European Society for Blood and Marrow Transplantation (EBMT) registry reported a clinical remission or significant symptomatic improvement after AHSCT rate of up to 68% in CD, with a median follow-up time of 41 months.[6]

Safety is the major limiting factor in the implementation of AHSCT for a broader patient population. AHSCT is, indeed, associated with an overall mortality rate of up to 2% and a high rate of adverse events (AE), mainly infections, related to the use of high-dose immunosuppressive agents, especially cyclophosphamide (Cy), during the mobilisation and conditioning regimens.[7–9] In this regard, improvements have been made by implementing safety protocols to control and reduce potential AEs.[10] In 2019, efforts to enhance transplant safety protocols and address the design limitations of the previous ASTIC trial led to the initation of a multicenter, parallel-group, randomized controlled trial called AsticLITE.[11] This trial aimed to enhance transplant safety by utilizing lower doses of Cy both during mobilisation and conditioning, along with reduced doses of anti-thymocyte

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globulin (ATG) during conditioning. Additionally, it aimed to address previous limitations by incorporating endoscopic remission as a more realistic and rigorous endpoint. However, this protocol also introduced changes, such as repeated dosing with fludarabine at 25 mg/m$^2$ during the conditioning regimen and a second round of granulocyte colony-stimulating factor (G-CSF) treatment on day 5 of stem cell infusion. After enrolling the initial 23 patients (13 cases and 10 controls), this study was prematurely halted due to the high incidence of AEs, particularly unexpected severe AEs observed in the treated group. These AEs may have been, at least partially, attributed to the administration of additional drugs.

As Cy is the main responsible for toxicity during mobilization, the exploration of alternative protocolos is justified. A novel mobilization protocol has been developed, eliminating completely the use of Cy and relying solely on G-CSF with the option of incorporating plerixafor (AMD 3100) in case of inadequate mobilization.

The objective of the present study was to assess the impact that eliminating Cy during mobilisation, while maintaining the currently recommended conditioning regimen, could have on the safety and efficacy of AHSCT in patients with refractory CD. We hypothesize that removing Cy during mobilisation should reduce the rate of AEs during this phase, thereby increasing the overall safety of the transplant. Clinical and endoscopic responses, as well as changes in peripheral immune cells and the humoral compartment, were monitored in these patients to assess the impact that the Cy-free mobilisation protocol could have on the transplantation's efficacy.

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## MATERIAL AND METHODS

### Study design

A prospective, open, single-centre, non-interventional study was conducted at Hospital Clinic Barcelona from June 2017 to October 2022 to assess the safety and efficacy of AHSCT with a Cy-free mobilisation regimen in patients with refractory CD. Main inclusion criteria were as follows: 1) confirmed diagnosis of active CD at the time of inclusion defined as Crohn's Disease Activity Index (CDAI) >220 or objective evidence of active disease as detected by ileo-colonoscopy and/or Magnetic Resonance-enterography (MRE); 2) unsatisfactory disease course despite the use of at least 2 immunosuppressors (thiopurines and methotrexate), 2 anti-TNF agents (infliximab and adalimumab), vedolizumab and ustekinumab. Full eligibility criteria are outlined in **Supplementary Table 1**. Discontinuation of concomitant immunosuppressive therapy before mobilisation was mandatory, with the following wash-out periods: 8 weeks for biologic agents, 2 weeks for azathioprine/6-mercaptopurine, and 1 week for all other immunosuppressive treatments. Ongoing steroid therapy was permitted at the discretion of the treating physicians based on their clinical judgment.

### Ethical issues

AHSCT in patients with refractory CD was approved as a therapy by the local transplantation organization OCATT (Organització Catalana de Transplantaments). The study was conducted in accordance with the Declaration of Helsinki (Fortaleza, Brazil, October 2013). The current protocol was approved by the Ethics Committee of the Hospital

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Clinic of Barcelona (HCB/2017/0594). All patients received adequate and extensive counselling and provided signed informed consent.

**Objectives**

Primary objectives: 1) to assess the safety of a Cy-free mobilisation regimen based on G-CSF +/-Plerixafor; 2) to assess the global efficacy of AHSCT following a Cy-free mobilisation regimen, as measured by clinical remission (CDAI <150) and/or clinical response (reduction of >100 points of basal CDAI) at weeks 13, 26, 52, and the last follow-up.

Secondary objectives: 1) to assess endoscopic remission (defined as Simple Endoscopic Score for Crohn's disease [SES-CD] ≤2) or response (>50% decrease of basal SES-CD) at weeks 26, 52 and the last follow-up; 2) to identify immunological and transcriptional changes related to AHSCT in blood and intestinal biopsies.

**Pre-mobilisation assessments**

Before mobilisation, in addition to endoscopic and radiological evaluations to confirm CD activity, patients underwent a comprehensive medical assessment. This included bone marrow aspirate, left ventricle ejection fractioning, pulmonary function test, dental evaluation, and bone densitometry (DEXA scan). Potential latent infections were ruled out, including cytomegalovirus (CMV), herpes simplex virus (HSV), varicella-zoster virus (VZV), Epstein-Barr virus (EBV), human T-lymphotropic virus type 1 and 2, hepatitis viruses,

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human immunodeficiency virus (HIV), *Toxoplasma gondii* and tuberculosis. Fertility preservation procedures were performed when deemed appropriate.

**Cyclophosphamide-free mobilisation protocol**

Mobilisation was conducted on an outpatient basis, with patients receiving subcutaneous G-CSF at a dosage of 12 μg/Kg/day for up to 5 days. In the event of inadequate mobilisation (<3x10$^6$ CD34+ cells/Kg), a single dose of 240 mg plerixafor (AMD 3100) was administered. Apheresis was conducted using a Cobe Spectra System with Mononuclear cell software (Terumo BCT, Lakewood, Co, USA). This involved double-strength anticoagulant citrate dextrose solution A at a rate of up to 2.4 mL/L/min, with routine prophylactic calcium and magnesium infusion (1 mol calcium per every 10 mol citrate). For harvesting, the minimum amount of CD34+ cells mobilized and extracted was 3x10$^6$ unselected CD34+/Kg and, when possible, an additional amount of 2x10$^6$ CD34+/Kg cells were collected as back-up. Haematopoietic stem cells (HSC) were cryopreserved in dimethyl sulfoxide (DMSO) at 10% concentration until transplantation. The main differences in Cy-based mobilisation regimens are summarized in **Table 1**.

**Conditioning and follow-up**

A non-myeloablative conditioning regimen, consisting of Cy 50 mg/kg/daily for 4 days and rabbit-ATG 2.5 mg/kg/daily for 3 days, was administered, following current recommendations.[6,10] A 500 mg dose of intravenous methylprednisolone was added for 3

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days to reduce the AEs associated with rabbit-ATG treatment. Harvested HSCs were finally infused, and engraftment was confirmed by haematologic recovery, which was defined as an absolute neutrophil count >0.5x10$^9$/L and a platelet count >20x10$^9$/L for at least 3 consecutive days.

During conditioning and transplantation, supportive care was provided, including hospitalization in isolated rooms equipped with high-efficiency particle-arresting filters and antimicrobial prophylaxis targeting the most common infectious agents, including *Pneumocystis jiroveci* and HSV. Prophylaxis was maintained until immune system recovery, and antifungal prophylaxis was continued until neutrophil recovery (>500 cells/mm$^3$). During the aplasia period, patients were put on parenteral nutrition. A low microbial diet was maintained until CD4 recovery (>400 cells/mm$^3$). When appropriate, patients received irradiated transfusions of red cells or platelets, and then only if prolonged neutropenia (>14 days), G-CSF was administered. After discharge, patients were followed up on a regular basis: at weeks 13 (T13), 26 (T26), 39 (T39) and 52 (T52) from the day of HSC infusion and colonoscopy and/or MRE were performed at T26, T52, week 106 (T106) and week 236 (T236) or whenever clinically indicated.

**Blood sample collection**

Blood samples were collected for all patients at baseline (T0; pre-mobilisation) and at different time points after AHSCT for up to 2 years of follow-up (T13, T26, T52, T80 and T106). For blood mRNA analysis, blood was collected into PAXgene tubes and frozen at -20ºC (PreAnalytiX [Qiagen, Spain]). A second blood sample was collected to obtain serum

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for antibody determination (**Supplementary File**). An additional 40 ml of blood was used to isolate peripheral blood mononuclear cells (PBMCs). PBMCs were cryopreserved until later use for cell population analysis using flow cytometry.

**Collection and analysis of intestinal biopsies**

Intestinal biopsies were collected at the described time points from areas of currently or previously inflamed mucosa. Biopsies from healthy non-inflammatory bowel disease controls undergoing endoscopy for colorectal cancer screening and showing no signs of inflammation or dysplasia were included for comparisons. All biopsies were placed in RNA-later RNA Stabilization Reagent (Qiagen) and stored at -80ºC for later RNA isolation.

Methods for flow cytometry analysis, RNA isolation, cDNA synthesis, Real-Time Polymerase Chain Reaction (RT-PCR), and serum antibody determinations are detailed in the **Supplementary File**.

**Statistical analysis**

Sample size was not calculated for this study. All consecutive patients treated according to the study protocol were included and analysed. Qualitative variables were described as frequencies and percentages, and quantitative variables as median and interquartile ranges. Comparisons between groups were performed using the Kruskal-Wallis test, with corrections for multiple comparisons made by controlling the False Discovery Rate (two-stage step-up method of Benjamini, Krieger and Yekutieli). P-values <0.05 were considered

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statistically significant. Analyses were performed with SPSS Statistics (IBM Corp. Released 2020. IBM SPSS Statistics for Windows, Version 29.0) and GraphPad Prism v. 9.1.0. (GraphPad Software, CA, USA).

## RESULTS

### Study population and demographics

A total of 14 patients were included in the study. The main demographic characteristics of the study population are presented in **Table 2**. Patients who received AHSCT presented complicated and highly refractory CD: more than 70% had stricturing disease, and 100% were refractory to at least four different biologics. In addition, 13 out of 14 had undergone at least one previous surgery. At baseline, 10 patients (71.4%) presented with a CDAI >220 along with endoscopic activity, while one patient showed objective evidence of severe intestinal inflammation at endoscopy despite having a normal CDAI. Additionally, two patients showed severe intestinal inflammation on MRE, despite having a normal CDAI, and one patient had an elevated CDAI in the context of multiple refractory postoperative recurrence. All patients underwent mobilisation and successful transplantation. A minimum 52-week follow-up was completed by all patients, and the median long-term follow-up was 106 weeks (range 52-348).

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**Mobilisation and conditioning outcomes and adverse events**

Cy-free mobilisation was successful in all patients (14/14). The median CD34+ cells recollected were 7.7 x$10^6$/Kg (3.7-34.5). In 7 patients (50%), G-CSF alone was not able to obtain sufficient amounts of CD34+ cells and Plerixafor was required according to the study protocol.

During mobilisation, one unrelated serious adverse event (SAE) was registered. One patient presented intestinal bleeding from the ileal site of the biopsy performed during the pre-mobilisation endoscopy. This patient completely recovered after hospitalization, red blood cell transfusion and endoscopic treatment. Two additional AEs were noted: one patient experienced fever related to a post-mobilisation scheduled ileocecal resection, and one patient experienced muscle pain. The full description of AEs is shown in **Table 3**, which has been compared with a previous series of patients from the same centre that were mobilized with Cy.[12] Of note, compared to the prior Cy-based protocol, hospitalization, antibiotic prophylaxis, or total parenteral nutrition within the aplasia period were not required during mobilisation, which was performed for all 14 patients on an outpatient basis.[12]

During conditioning, AEs included the following: febrile neutropenia (n=7), infections from *Brevibacterium* and *Stenotrophoomona malthophila* (n=2), systemic CMV reactivation (n=1) and HSV (n=1), ATG reactions (n=4), grade I oral mucositis (n=5), grade 1 gastrointestinal mucositis (n= 4) and serum sickness (n=1).

Median total cells infused were 4.1x$10^6$/Kg (3.0-8.6). All patients achieved successful transplant engraftment. Neutrophil and platelet engraftment occurred at 13 days (10-17) and 9 days (0-13), respectively. In 13 patients (92.8%), at least 1 blood transfusion (median blood

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units= 3) was required, and 12 patients (85.7%) required at least 1 platelet transfusion (median platelet units= 3).

**Efficacy of AHSCT after Cyclophosphamide-free mobilisation**

Drug-free clinical remission rates were 78.6% at week 13, 57.1% at week 26, 21.4% at week 52, and 7.1% at the last follow-up (median of 110 weeks).

When considering clinical remission regardless of biologic therapy or steroid use, the rates were 78.6%, 71.4%, 64.3%, and 64.3% at the same respective time points (**Figure 1a**).

In the overall patient cohort, the median CDAI scores at baseline, week 52, and at the last follow-up were 196 (144-261), 109 (87-241), and 98 (77-201), respectively. Importantly, there was a significant decline in CDAI scores between baseline and weeks 13 (p=0.018) and 26 (p=0.047) (**Figure 1b)**.

Twelve patients required reintroduction of biologics during follow-up, consisting of anti-TNF (n=5), Ustekinumab (n=3), and Vedolizumab (n=4), while 8/12 patients (66.6%) achieved clinical remission. The median time for reintroducing biologics after transplantation was 45.5 weeks (26-106).

For the 12 patients who underwent endoscopy follow-up, endoscopic remission and response were 41.7% and 58.3% at week 26, 25% and 33.3% at week 52, and 16.7% and 41.5% at the last follow-up (**Figure 1c**). The median SES-CD was 12 at baseline, 3 at week 26, 9 at week 52, and 6 at the last follow-up (p=0.123) (**Figure 1d**).

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**Safety of AHSCT following Cy-free mobilisation**

During follow-up, 15 AEs were reported in 10 patients (71.4%), including 2 SAEs (28.6%). A full description is provided in **Table 4**. A 48-year-old man died at week +102; he had been diagnosed with ileal adenocarcinoma 10 years before and was disease free at the time of transplant. However, one year after transplant he presented a cancer recurrence and underwent ileocolonic resection with subsequent peritoneal carcinomatosis. A 45-year-old man with a colostomy underwent scheduled surgery for colonic transit reconstruction at week 52; a rectal perforation occurred during surgery and was successfully treated with no further sequelae.

**Changes in peripheral leukocytes following AHSCT**

As anticipated, the conditioning regimen induced neutropenia (neutrophil counts <0.5x $10^9$/L) in all patients, with neutrophil engraftment occurring around 2 weeks. Nevertheless, the number of neutrophils in the blood remained significantly lower than at baseline for up to 13 weeks after AHSCT (**Figure 2A**). Importantly, this decrease in neutrophil counts was comparable to that observed in patients undergoing Cy-based mobilisation in a previous cohort.[12] While the total lymphocyte count did not significantly change after AHSCT in patients who received Cy-free mobilisation, the number of CD4 T cells, but not CD8 or CD19 B cells, significantly decreased for up to 1 year (**Figure 2B**), consistent with our previous findings in the Cy-based mobilisation cohort[19]. Additionally, as with the

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conventional protocol, the $CD4^+CD45RA^+$ compartment was the most significantly depleted following AHSCT (**Figure 2C**). Further analysis revealed that naïve ($CD4^+CD45RA^+CD62L^+$), terminally differentiated effector memory re-expressing CD45RA (TEMRA; $CD4^+CD45RA^+CD62L^-$) and central memory ($CD4^+CD45RA^-CD62L^+$) T cells were all significantly decreased, while the abundance of effector memory CD4 cells ($CD4^+CD45RA^-CD62L^-$) remained unchanged following the conditioning regimen (**Figure 2C and 2D**). Nonetheless, AHSCT significantly reduced the expression of IL-7R within effector ($CD4^+CD45RA^-CD62L^-$) and central ($CD4^+CD45RA^-CD62L^+$) memory cells (**Figure 2E**).

As shown in **Figure 2B**, the overall CD8 compartment was not affected; however, the number of naïve ($CD8^+CD45RA^+CD62L^+$) cells was significantly reduced (**Supplementary Figure 1A**). Furthermore, IL-7R expression was significantly downregulated in all four CD8 compartments, showing that AHSCT can affect the phenotype of circulating lymphocytes (**Supplementary Figure 1B**). In contrast to the changes observed in IL-7R, no changes were detected in CD28 surface expression in any CD4 or CD8 subsets following ASCHT (data not shown). We also observed a decrease in the number of memory $CD19^+CD27^+$ B cells and plasmablasts ($CD19^+CD27^+IgD^-CD38^+$) following AHSCT (**Figure 2F**), which is in agreement with our previous observations.[13]

**Whole blood transcriptional changes following AHSCT**

Blood mRNA expression confirmed that *IL7R* and *PTK7* (expressed by recent thymic emigrants) and *CCR7*, a marker predominantly expressed in naive $CD4^+$ T cells, were downregulated, while *CD28*, a marker of activated memory $CD4^+$ T cells, did not exhibit any variations following AHSCT, thus corroborating our flow cytometry data (**Supplementary**

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**Figure 2**). Within the B cell compartment, expression of *CD79A* or *IGHD* did not significantly change, confirming the limited changes detected within this subset in peripheral blood.

**Serum antibody levels following AHSCT**

Similar to data reported for patients who underwent a Cy-based mobilisation, total IgA and IgM in serum showed a trend towards decreased concentrations at early time points after AHSCT, while total IgG and specific IgG for *C. tetani* or *Rubella* did not show any variations (**Figure 2G**).[12]

**Transcriptional changes in the intestinal mucosa following AHSCT**

Total RNA from a reduced number of patients with ileal involvement was available at different time points during the transplant protocol. Expression of several genes, including the T-cell pan-markers *CD3E* and *CD28*, markers of resident T cells *ITGAE*, *CD69* and *ITGA1* or Treg *IL2RA*, B cells *CD79A*, plasma cells *DERL3*, the inflammatory cytokine *CXCL10* and the neutrophil marker *PROK2*, were all measured and are shown in relation to the efficacy of the AHSCT protocol (**Figure 3).** Although no statistical analysis could be performed due to the low number of individuals per group, we observed a trend towards decreasing T-cell markers regardless of response. In contrast, changes in inflammation-related markers as diverse as *IL2RA*, *CXCL10*, *DERL3* and *PROK2* mirrored treatment efficacy.

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## DISCUSSION

The present study shows the enhanced safety and sustained efficacy of a Cy-free mobilisation regimen based on G-CSF, with or without Plerixafor, in AHSCT for refractory CD.

Over the past 25 years, AHSCT has increasingly been used for the treatment of patients with refractory inmunomediated diseases such as multiples sclerosis, systemic sclerosis, inflammatory arthritis, and systemic lupus erythematosus.[14] Despite the availability of modern biologic and targeted synthetic disease-modifying therapies, the annual numbers of HSCT transplants continue to rise. This trend reflects the ongoing clinical need for effective therapies in specific clinical conditions or individual cases. Regarding refractory CD, AHSCT has shown efficacy, albeit with a significant incidence of AEs, including some serious ones, as outlined in **Table 5.** AEs in the context of HSCT for refractory CD are frequently associated with high-dose chemotherapy, including Cy during mobilisation and conditioning, thereby limiting its applicability to a wider patient population. In 2019, a multicentre, parallel-group, randomised controlled trial (AsticLITE) was conducted, using low-dose Cy and G-CSF mobilisation, along with a reduced-intensity conditioning regimen.[11] However, this was terminated early due to a high incidence of AEs, particularly unexpexted severe AE, mainly related to one case of pulmonary veno-oclusive disease and three cases of histologically confirmed renal thrombotic microangiopathy (TMA). Moreover, one patient died from respiratory insufficiency and another patient from acute oliguric renal failure, potentially associated with TMA (although not proven by histology assessment). It is suggested that CD patients, owing to their prior exposure to

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immunomodulators and biologics, may constitute a high-risk cohort, or alternatively, the conditioning regimen, which employed a combination of fludarabine and ATG, could be associated with microangiopathy and subconsequent severe renal impairment. In response to this lack of success, an accompanying editorial advised against AHSCT in CD patients based on the principle of "first, do not harm".[15] Indeed, due to suboptimal outcomes associated with specific protocols and the elevated incidence of AE, the use of AHSCT in CD is debated among experts. Some authors argue against its utilization, considering it an imbalanced treatment option given the higher AE rate compared to medical therapies. Consequently, they dismiss AHSCT as a viable therapy for CD altogether. However, the authors of the present study argue that it is crucial to weigh the associated morbidity and mortality in patients with refractory CD before disregarding a treatment option that has shown efficacy in clinical practice.

The present Cy-free mobilisation regimen has shown an excellent safety profile, with a drastic reduction of AEs. Muscle pain, a commonly known AE related to G-CSF, was the only significant event observed during mobilisation.[16] Notably, unlike previous mobilisation regimens requiring an average of 19 days of hospitalization, no patients required hospitalisation. The Cy-free mobilisation protocol also eliminated the need for antibiotic prophylaxis, parenteral nutrition, and other specific safety measures previously implemented.[3]

Patients under the Cy-free protocol were treated as outpatients, and successful mobilisation was achieved in all cases. The removal of Cy has been associated with insufficient mobilisation of progenitor cells;[17] hence, our protocol incorporated the use of Plerixafor as

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a safe alternative for patients unable to mobilize sufficient amounts of CD34+ cells. Plerixafor is a CXCR4 chemokine receptor antagonist administered subcutaneously in combination with G-CSF to achieve an adequate mobilisation of stem cells to the peripheral blood, particularly in individuals with poor mobilization.[18] It is generally well tolerated, with a low rate of mild AEs. Despite being required by 50% of patients, the concomitant use of Plerixafor did not lead to an increase in AEs or hospitalization. The rate of Plerixafor use in this case series was higher than in previous reports, which was likely due to the extended history of immunosuppressive therapies in our patient cohort.[19,20] Despite concerns about mobilisation with G-CSF alone, which induced relapses in some immune-mediated diseases, no such observations were made in this study or in previous series of patients who had undergone Cy-free mobilisation.[21,22]

Regarding conditioning and transplantation protocols, no significant differences or new unexpected AEs were noted csompared to prior reports, including the series of patients transplanted in our center and submitted to an identical protocol.[12,23] The major issue during conditioning involved infectious AEs related to the use of chemotherapy. Engraftment was successfully achieved in all cases.

After AHSCT, 10 patients experienced at least 1 AE. The SAE rate was 28.6%, consistent with global safety data on AHSCT.[9] In the present cohort, one patient died from a metastatic ileal adenocarcinoma diagnosed one year after transplantation. Ileal adenocarcinomas are rare malign tumours of the small bowel, exhibiting a 60-fold higher incidence in patients with CD. This heightened incidence is partly attributed to the carcinogenic effects of chronic

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inflammation and partly to the long course of immunosuppression used in patients with refractory CD.[24]

The observed AHSCT efficacy was comparable to previous studies, with a notable drug-free remission rate of 57% at 6 months, gradually decreasing to 21.4% at one year and 7.1% at the last follow-up (110 weeks). Disease activity, as measured by the CDAI score, significantly decreased from baseline to last-follow-up (196 vs 98, $p<0.05$). In a previous case-series from our group, we reported higher rates of drug-free remission (70%, 61% and 47% at 6 months, 1 year and 3 years, respectively). These variations could be attributed to significant differences in the current study populations, including higher rates of ileal involvement (21% vs 3%) and stricturing disease (71.4 vs 10%), resulting in a lower response to therapies, and, consequently, a higher degree of refractoriness.[12,25] Notably, in prior studies, patients were enrolled based on their lack of response to anti-TNF and immunosuppressive agents, while in the present study, refractoriness was defined as no response to anti-TNF, ustekinumab, or vedolizumab.[5,12]

The efficacy of AHSCT is supported by endoscopic findings, showing a response rate of 58.3% and a remission rate of 41.7% at 6 months, which decrease to 33% and 25% respectively after one year. These rates mirror the variations in clinical symptoms and the progressive worsening over time. It is crucial to place these results in the context of the current medical landscape. For instance, in the FORTIFY trial, risankizumab, a selective interleukin-23 p19 inhibitor, showed an endoscopic response rate of 46.8% and a remission rate of 29% at one year in a patient population where 72% had prior exposure to one or more biologics.[35] Similarly, the U-ACHIEVE trial demonstrated that the Janus kinase inhibitor

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upadacitinib achieved response and remission rates of 49% and 21.9% at one year, respectively, in a population where 48% had prior biologic exposure.[36] While endoscopic response and remission rates may seem comparable to those of AHSCT, it is crucial to interpret these figures with caution since they apply to patients who have experienced one or sometimes two biologic failures and may have a shorter disease duration, implying a potentially less refractory disease course.

As previously described, patients who had lost response were able to achieve clinical remission with the reintroduction of biologic therapies.[12] Indeed, our data show that 66.6% of patients were in clinical remission at the last follow-up after the reintroduction of biologic therapy.

In a previous study, we described, for the first time in CD, the changes induced by AHSCT in peripheral and intestinal immune populations.[13] Our findings revealed that AHSCT induces sustained depletion in the naïve CD4 compartment, and an expansion in circulating naïve B cells. Importantly, we found no association between the intensity of these changes and the efficacy of the transplant in terms of clinical or endoscopic remission. Hence, we argued that increasing immune depletion by subjecting patients to more severe conditioning would not result in increased response rates. Instead, we directed our efforts towards enhancing the safety of the protocol while maintaining efficacy.[10] As demonstrated here, maintaining the conditioning regimen (after Cy-free mobilisation) ensured that changes in peripheral and intestinal CD4 T cells and memory B cells were sustained, both in blood and in intestinal tissues. Remarkably, this study confirms our previous observation that the

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conditioning used in CD, while reducing total IgA and IgM levels in the blood, has no effect on the total IgG or serum levels of protective IgG antibodies.[13]

The main limitations of this study include the small sample size and the single-centre design. Moreover, although all patients completed a minimum 52-week follow-up, long-term data are limited, potentially affecting the efficacy assessment.

In summary, we assert that the novel Cy-free mobilisation regimen for AHSCT in CD is safe, as it significantly reduces AEs during mobilisation, thereby enhancing the overall feasibility of the therapy, without compromising efficacy. As previously described, although a gradual loss of response is observed after AHSCT, a substantial proportion of patients responded to therapies that were previously ineffective. Further studies are necessary to explore the effects of early reintroduction of drugs on relapse rates after transplantation and the overall long-term efficacy of this strategy.

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## ACKNOWLEDGEMENTS

We would like to thank Marta Martinez for her assistance with the PCR analysis and Marc Buendia for developing applications that facilitated the data analysis.

## COMPETING INTERESTS

AG received support for meetings and travel from Galápagos, Janssen, Ferring and Dr. Falk. ML has received research support from Terumo BCT, Macopharma and Sanofi-Genzyme; speaker honoraria from Grífols and Terumo BCT and fees as a consultant for Grífols, Fresenius Kabi, Cerus and Macopharma. IO has served as speaker and/or consultant and has received educational funding from MSD, Abbvie, Pfizer, Takeda, Janssen, Faes Farma and Chiesi, and has received research funding from Abbvie and Faes Farma. AFC has served as a speaker, or has received education funding from Dr. Falk, Janssen, Takeda, Chiesi and Pfizer.

JP received consultancy fees/honorarium from AbbVie, Alimentiv, Athos, Atomwise, Boehringer Ingelheim, Celsius, Ferring, Galapagos, Genentech/Roche, GlaxoSmithKline, Janssen, Mirum, Nimbus, Pfizer, Progenity, Prometheus, Protagonist, Revolo, Sanofi, Sorriso, Surrozen, Takeda, and Wasserman. AS has received grants from Pfizer, Roche-Genentech, Abbvie, GSK, Scipher Medicine, Alimentiv, Inc, Boehringer Ingelheim and Agomab; received consulting fees from Genentech, GSK, Pfizer, HotSpot Therapeutics, Alimentiv, GoodGut and Agomab. ER has received support for congress and conference attendance, speaker fees, research support and/or consulting fees from MSD, Abbvie,

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Ferring, Janssen, Otsuka, Pfizer, Takeda, Galapagos, Kern Pharma and Fresenius Kabi. The rest of the authors have no conflict of interest to declare.

## AUTHORS' CONTRIBUTIONS

AG, MR, MV, AS, ER: initial draft of the manuscript and data analysis. MR, AS, ER: study design. RB, PM, CM, FFA, MSL, AD, AS, ML, JC, IO, AFC, BC, BG, AV, MCM, AG, IT, ME, AMC: data collection and management. AG, JP, AS, ER: manuscript review and editing.

## FUNDING

The present study received financial support from the Instituto de Salud Carlos III-Ministerio de Ciencia e Innovación PI17/00513.

## DATA AVAILABILITY

The data underlying this article cannot be shared publicly due to data protection regulation. The data will be shared on reasonable request to the corresponding author.

Manuscript Doi: 10.1093/ecco-jcc/jjae076

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**FIGURE LEGENDS**

**Figure 1.** Efficacy of AHSCT at different time points during follow-up. A) CD clinical remission or activity with and without biologic drugs is shown as the percentage of patients. B) Clinical activity according to CDAI. C) Endoscopic remission and response are shown as percentage of patients. D) Endoscopic activity according to SES-CD. In all graphs, time points express the number of weeks after AHSCT up to the last follow-up. *$p<0.05$ compared to T0 by the Kruskal-Wallis test and corrected by FDR.

**Figure 2.** Blood cellular and humoral immune compartments after AHSCT. A) Neutrophil and lymphocyte numbers (x10^9 cells/L) after AHSCT in the previous cy-based cohort (grey) and in the current cy-free patient cohort (black). B) Number (cells/ul) of CD4+, CD8+ and CD19+ lymphocytes. C) Flow cytometry dot plots of one representative patient pre-mobilisation (T0) and during different follow-up time points. CD4+ T cells are shown based on the expression of CD45RA and CD62L markers that define 4 populations of naïve (CD45RA+CD62L+), terminally differentiated effector memory (TEMRA, CD45RA+CD62L-), effector memory (EM, CD45RA-CD62L-) and central memory (CM, CD45RA-CD62L+) T cells. D) Number (cells/ul) of CD4+ T cell subpopulations. E) Mean fluorescence intensity (MFI) for IL7R in CD4+ subpopulations. F) Number (cells/ul) of CD19+ B cell subpopulations. G) Amount of total IgA, IgM and IgG (g/L) and specific IgG (UI/ml) for *C.*

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*tetani* and *Rubella* determined in the serum of patients. The dotted lines represent the serological protection level for total immunoglobulins and the threshold for the presence of protective IgG in the case of specific IgG for *C. tetani* and *Rubella*. In all graphs, time points express the number of weeks after AHSCT up to 1 (T52) or 2 years (T106) of follow-up. *p<0.05, **p<0.01, ***p<0.001; ****p<0.0001 compared to T0 by the Kruskal-Wallis test and corrected by FDR.

**Figure 3.** qPCR analysis of intestinal ileal biopsies showing mRNA expression of Tcell (*CD3*, *CD28*, *ITGAE*, *CD69*, *ITGA1* and *IL2RA*), B and plasma cell (*CD79A* and *DERL3*), inflammation (*CXCL10*) and neutrophil (*PROK2*) related genes. The Grey square at T0 shows the expression in healthy controls (HC) (n= 10-14). Closed circles and opened triangles show ileal biopsies with active (non-responder) or inactive (responder) inflammation, respectively, at the time of endoscopy (for T26 and T52) and at T52 for T0. The low number of patient samples per group (T0 n=3/4 [active/inactive], T26 n=2/2, T52 n=2/2-3) did not allow for the application of any statistical test. Data are shown as Arbitrary Units (AUs) mean±standard error of mean (SEM).

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**TABLES**

**Table 1. Main differences related to Cy-based and Cy-free mobilisation regimens.**

| **Cy-based mobilisation**[10] | **Cy-free mobilisation** |
|---|---|
| Inpatients | Outpatients |
| Antibiotic prophylaxis | No specific prophylaxis |
| Parenteral nutrition | Oral nutrition |
| Cy (2g/m²/day for 2 days) + G-CSF | Only G-CSF |

Cy: cyclophosphamide; G-CSF: granulocyte colony-stimulating factor

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**Table 2. Study population demographics and disease characteristics**

| | |
|---|---|
| **N** | **14** |
| **Females, n (%)** | **9 (64)** |
| **Age at enrolment, median years (range)** | **40 (20-48)** |
| **Disease duration, median years (range)** | **14 (3-37)** |
| **Location, n (%)** | |
| Ileal | **3 (21.4)** |
| Colonic | **1 (7.1)** |
| Ileocolonic | **10 (71.4)** |
| **Disease phenotype** | |
| Inflammatory | **2 (14.3)** |
| Stricturing | **10 (71.4)** |
| Penetrating | **2 (14.3)** |
| **Perianal disease, n (%)** | **2 (14.3)** |
| **NOD2/CARD15 gene mutation** | **1 (7.1)** |
| **Smoking history, n (%)** | |
| Smoker | **4 (28.5)** |
| Former smoker | **7 (50)** |
| Never smoker | **3 (21.4)** |
| **Prior therapies, n (%)** | |
| Thiopurines | **14 (100)** |
| Methotrexate | **14 (100)** |

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| | |
|---|---|
| Other immunosuppressive agents | **2 (14.3)** |
| Infliximab | **14 (100)** |
| Adalimumab | **14 (100)** |
| Vedolizumab | **14 (100)** |
| Ustekinumab | **14 (100)** |
| **Prior surgery for CD, n (%)** | **13 (93)** |
| **Ostomy at enrolment, n (%)** | **4 (28.5)** |
| **Basal SES-CD score, median points (IQR)** | **12 (2-23)** |

**CD Crohn's disease, SES-CD Simplified Endoscopic Activity Score for Crohn's disease**

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**Table 3. Adverse events during Cyclophosphamide-free mobilisation compared to a prior Cyclophosphamide-based mobilisation protocol**[12])

| Adverse events | Cy + G-CSF (n=29) | G-CSF ± Plerixafor (n=14) |
|---|---|---|
| Hospitalization, days, median (min-max) | 18.5 (14-73) | 0 |
| Neutropenia (N<0.5x10⁹/L), days, median (min-max) | 5 (2-7) | 0 (0-0) |
| Febrile neutropenia, no. (%) | 16 (62%) | 0 (0%) |
| Infectious complications, no. (%) | | |
| Bacteraemia with fever* | 1 (4%) | 0 (0%) |
| Bacteraemia with septic shock** | 2 (8%) | 0 (0%) |
| Labial herpes | 1 (4%) | 0 (0%) |
| Non-infectious complications, no. (%) | | |
| Renal failure*** | 1 (4%) | 0 (0%) |
| Adverse reaction to vancomycin | 1 (4%) | 0 (0%) |
| Red blood cell transfusion requirements, no. (%) | 15 (43%) | 1 (7%) |
| Arthromyalgia, no. (%) | 0 (0%) | 1 (7%) |
| Acute severe digestive bleeding, n (%) | 0 (0%) | 1 (7%) |

* Piperacillin-tazobactam-resistant *Escherichia coli* (blood culture).

** One case of piperacillin-tazobactam-resistant *Klebsiella pneumoniae* (catheter culture), and one case of extended-spectrum β-lactamase-producing *Klebsiella Pneumoniae* (blood culture).

*** Sepsis and nephrotoxicity related.

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**Table 4. Adverse events during follow-up after AHSCT**

| Description | Events n=14 |
|---|---|
| - Infections | |
| Bacterial infection | 3 |
| *Candida spp* infection | 2 |
| *Herpes Simplex Virus* reactivation | 2 |
| - Renal colic | 2 |
| - Intestinal surgery for disease control during follow-up | 2 |
| - Endoscopic balloon dilation of an ileocolonic anastomosis stricture | 1 |
| - Visible haematuria | 1 |
| - Appearance of a peristomal fistula | 1 |
| - Appearance of newly diagnosed perianal disease | 1 |
| - SAE | 2 |

SAE Serious adverse event

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**Table 5. Relevant clinical studies evaluating efficacy and safety of AHSCT for refractory CD.**

| AUTHOR; STUDY DESIGN | YEAR | PATIENTS | MOBILIZATION PROTOCOL | CONDITIONING PROTOCOL | CLINICAL REMISSION RATE | ADVERSE EVENT RATE | MORTALITY RATE |
|---|---|---|---|---|---|---|---|
| Oyama[26]; Clinical trial | 2005 | 12 | Cy + G-CSF CD34+ enriched | Cy 200 mg/Kg + equine ATG 90 mg/Kg | 91.6% (11/12) | 50% (6/12) | 0 |
| Cassinotti[27]; Clinical trial | 2008 | 4 | Cy + G-CSF | Cy 50 mg/Kg/day + rabbit ATG 2.5 mg/Kg | 100% (4/4) | 50% (2/4) | 0 |
| Burt RK et al[28]. Clinical trial | 2010 | 24 | Cy + G-CSF | Cy 200 mg/Kg + equine ATG 90 mg/Kg or rabbit ATG 6 mg/Kg | 100% (24/24) | 50% (12/24) | 5% (1) |
| Clerici M[29]; Clinical trial | 2011 | 6 | Cy + G-CSF | Cy 50 mg/Kg/day + rabbit ATG 2.5 mg/Kg/day | 100% (6/6) | 33.3% (2/6) | 0 |
| Hasselblatt P[30]; Clinical trial | 2012 | 9 | Cy 2g/m2/day + G-CSF 5mcg/Kg/day | Cy 50 mg/Kg/day | 55.5% (5/9) | 66.6% (6/9) | 0 |
| Snowden JA[31]; Retrospective observational study | 2014 | 6 | Cy 2-4g/m$^2$ + G-CSF 10mcg/Kg/day | Cy 200 mg/Kg + rabbit ATG 7.5 mg/Kg | 83.3% (5/6) | NA | 0 |
| Hawkey CJ[5]; Clinical trial | 2015 | 23 | Cy 2g/m$^2$ + G-CSF 10mcg/Kg/day | Cy 50 mg/Kg/day + rabbit ATG 2.5 mg/Kg/day | 8.7% (2/23) Sustained remission | 82.6% (19/23) | 4.3% (1) |
| Lindsay JO[32]; Retrospective observational study | 2017 | 40 | | | 38.5% (15/39) | 57.5% (23/40) | 2.5% (1) |
| Jauregui-Amezaga A[33]; Prospective Observationa | 2016 | 26 | Cy 2g/m$^2$ + G-CSF 10mcg/Kg/day | Cy 50 mg/Kg/day + rabbit ATG 2.5 mg/Kg/day | NA | 80.8% (21/26) | 5% (1) |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| l study<br><br>Lopez-Garcia A[12]; Prospective Observationa l study | 2017 | 29 | | | 70% (20/29) | 72.4% (21/29) | 3.4% (1) |
| Ruiz MA[34]; Clinical trial | 2017 | 14 | Cy 60mg/Kg + G-CSF 10mcg/Kg/day | Cy 50 mg/Kg/day + rabbit ATG 6.5 mg/Kg | 92.9% (13/14) | 28.6% (4/14) | 0 |
| Lindsay JO[11];<br><br>Clinical trial | 2024 | 13 | Cy 1g/m²+ G-CSF 5 mcg/Kg/day | Cy 60 mg/Kg/day + rabbit ATG 2.5 mg/Kg/day + fludarabine 25 mg/m²/day | 57% (4/7) | 100% (13/13) | 15.4% (2) |

Adverse events include related and not related complications after AHSCT. ATG: antithymocyte globulin; Cy: Cyclophosphamide; G-CSF: granulocyte colony-stimulating factor

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Figure 1

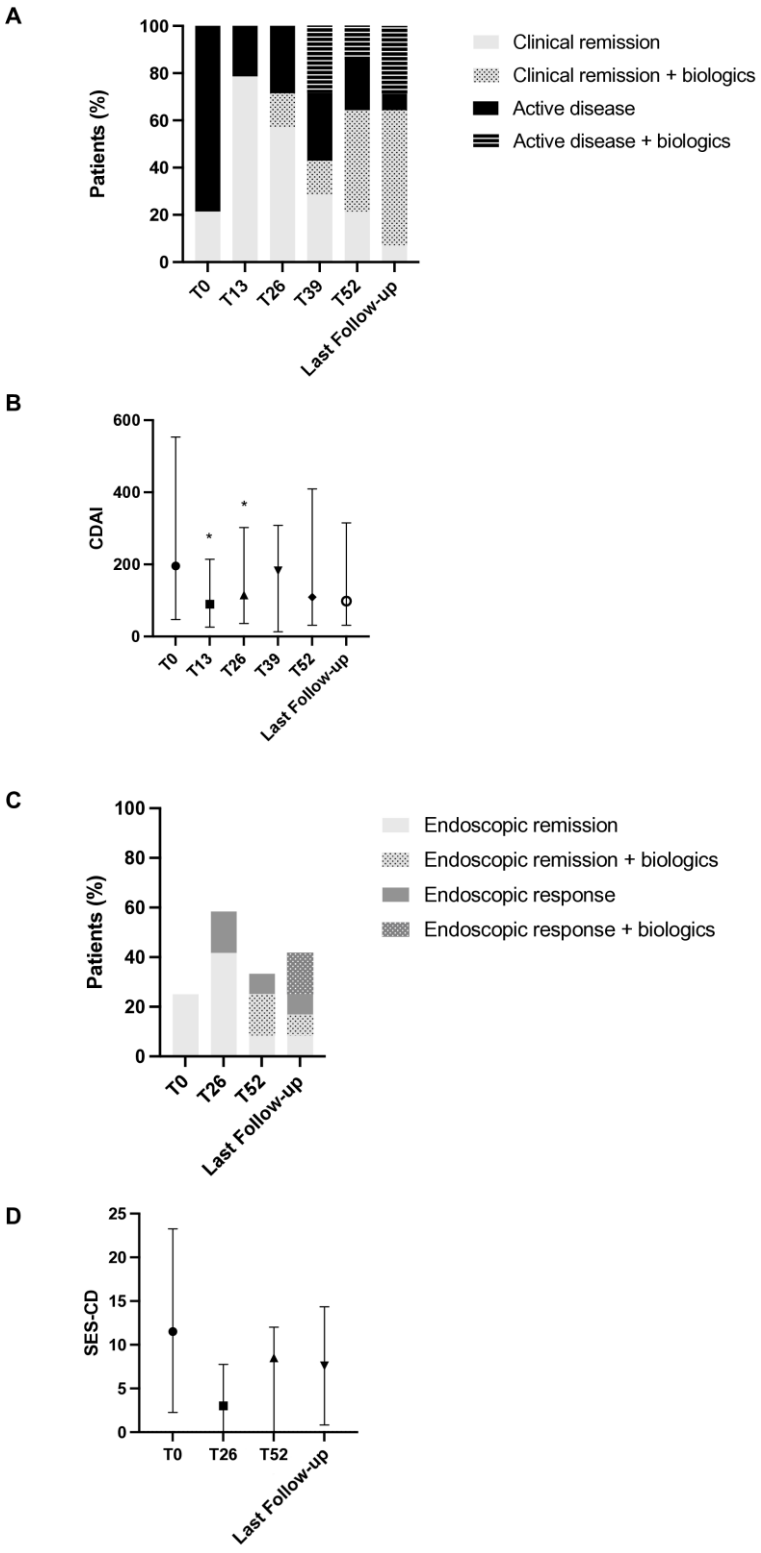

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Figure 2

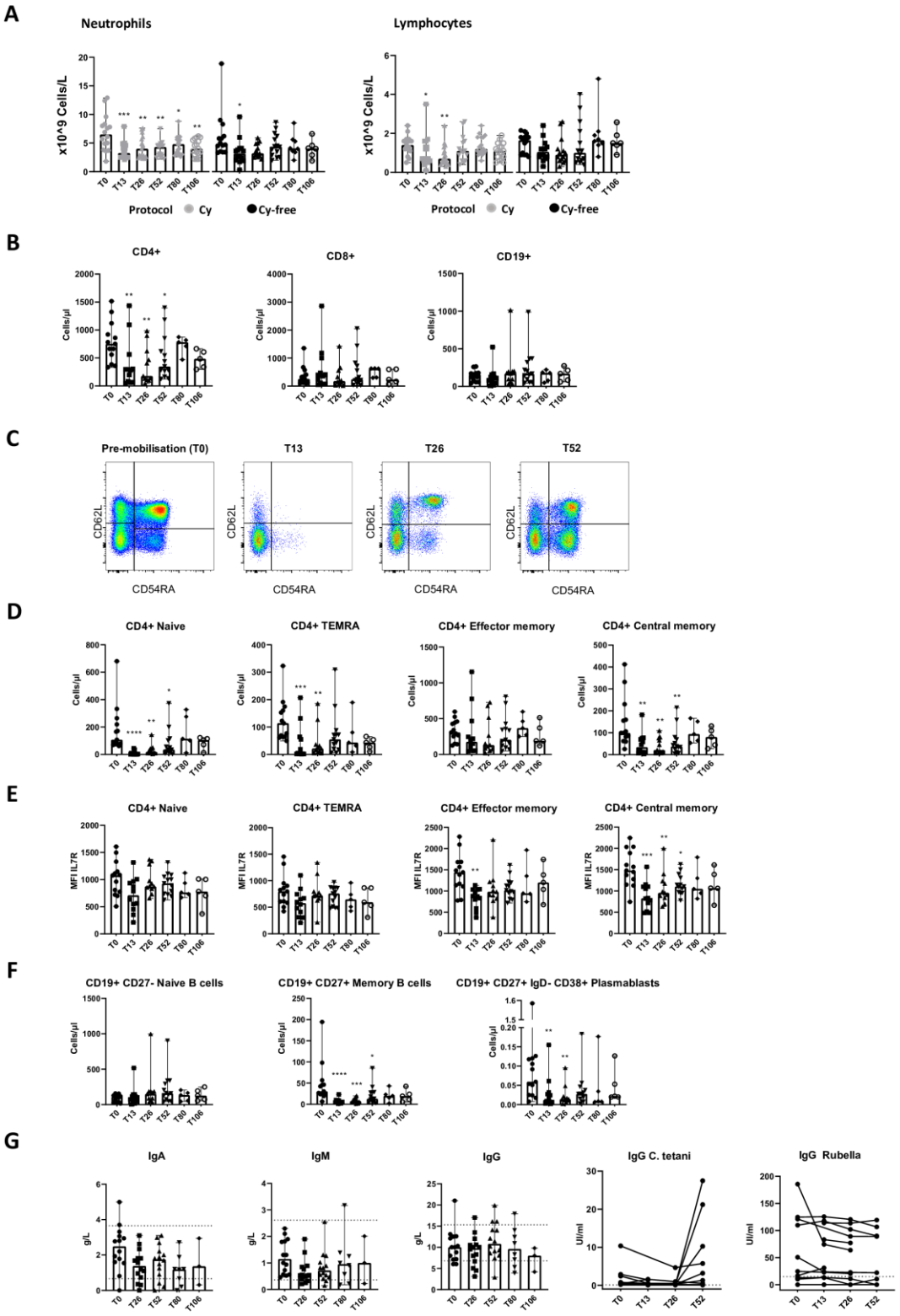

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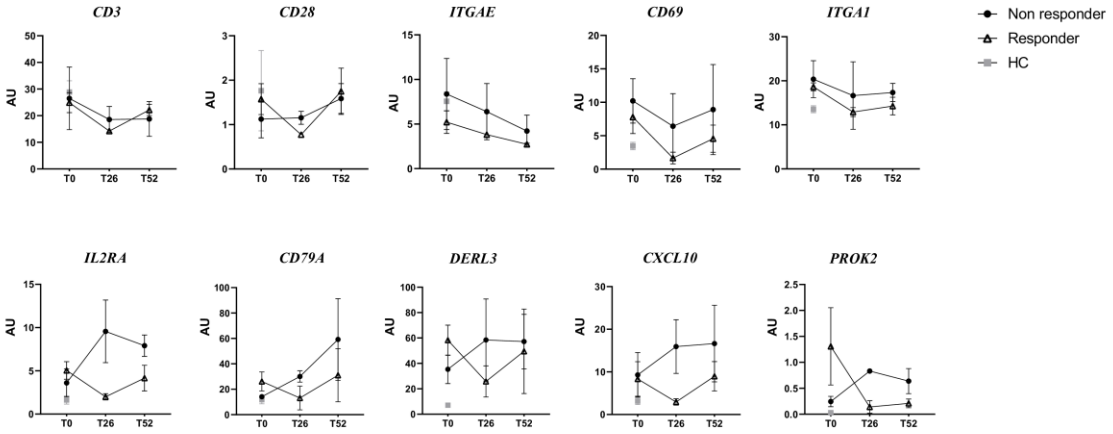
Figure 3



# Can we simplify the journey in UC?

## JYSELECA is a once-daily oral treatment* that provides rapid** and long-term† efficacy up to ~4 years[1–3]

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* Recommended dose for induction and maintenance is 200 mg once daily.[1] JYSELECA is not recommended in patients aged 75 years and older as there is no data in this population; in patients aged 65 years and over the recommended dose is 200 mg once daily for induction treatment and 100 mg daily for maintenance treatment.[1]
** Data from a *post-hoc* analysis of diary data from the double-blind, randomised, placebo-controlled 58-week SELECTION trial. Achievement of stool frequency subscore of ≤1 by Day 3 in biologic-naïve patients, and rectal bleeding subscore of 0 by Day 5 in biologic-experienced patients.[2]
† Interim analysis of SELECTIONLTE assessing the efficacy and safety of open-label JYSELECA 200 mg through LTE Week 144 in completers and LTE Week 192 in non-responders, respectively, representing a total of 3.9 years of treatment each (completers: 58 + 144 weeks; non-responders 10 + 192 weeks).[3]
†† Determined in a *post-hoc* exploratory analysis of the SELECTION trial assessing HRQoL and the comprehensive disease control multi-component endpoint, which comprises both clinical and QoL outcomes, in individuals receiving JYSELECA (n=786).[4] Each patient has their own definition of normal life.

▼ This medicine is subject to additional monitoring.

HRQoL, Health-related quality of life; LTE, Long term extension; QoL, Quality of life; UC, Ulcerative colitis.


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