Interim analysis

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Interim analysis: Open-label extension study of leniolisib for patients with APDS

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Background: Activated phosphoinositide 3-kinase delta (PI3Kδ) syndrome (APDS; or p110δ-activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency) is an inborn error of immunity caused by PI3Kδ hyperactivity. Resultant immune deficiency and dysregulation lead to recurrent sinopulmonary infections, herpes viremia, autoimmunity, and lymphoproliferation.
Objective: Leniolisib, a selective PI3Kδ inhibitor, demonstrated favorable impact on immune cell subsets and lymphoproliferation over placebo in patients with APDS over 12 weeks. Here, we report results from an interim analysis of an ongoing open-label, single-arm extension study.
Methods: Patients with APDS aged 12 years or older who completed NCT02435173 or had previous exposure to PI3Kδ inhibitors were eligible. The primary end point was safety, assessed via investigator-reported adverse events (AEs) and clinical/laboratory evaluations. Secondary and exploratory end points included health-related quality of life, inflammatory markers, frequency of infections, and lymphoproliferation.
Results: Between September 2016 and August 2021, 37 patients (median age, 20 years; 42.3% female) were enrolled. Of these 37 patients, 26, 9, and 2 patients had previously received leniolisib, placebo, or other PI3Kδ inhibitors, respectively. At the data cutoff date (December 13, 2021), median leniolisib exposure was 102 weeks. Overall, 32 patients (87%) experienced an AE. Most AEs were grades 1 to 3; none were grade 4. One patient with severe baseline comorbidities experienced a grade 5 AE, determined as unrelated to leniolisib treatment. While on leniolisib, patients had reduced annualized infection rates (P = .004), and reductions in immunoglobulin replacement therapy occurred in 10 of 27 patients. Other observations include reduced lymphadenopathy and splenomegaly, improved cytopenias, and normalized lymphocyte subsets.
Conclusions: Leniolisib was well tolerated and maintained durable outcomes with up to 5 years of exposure in 37 patients with APDS. ClinicalTrials.gov identifier: NCT02859727. (J Allergy Clin Immunol 2023;■■■:■■■-■■■.)

Key words: *APDS, primary immunodeficiency, PI3Kδ inhibitor, clinical trial, long-term safety, B cells, T cells, lymphoproliferation,* PIK3CD, PIK3R1

Activated phosphoinositide 3-kinase delta (PI3Kδ) syndrome (APDS; or p110δ-activating mutations causing senescent T cells, lymphadenopathy, and immunodeficiency) is an inborn error of immunity driven by PI3Kδ hyperactivity.[1,2] PI3Kδ homeostasis is critical for the development and function of both B and T cells.[3,4] PI3Kδ is composed of 2 subunits: the catalytic subunit p110δ (*PIK3CD*) and the regulatory subunit p85α (*PIK3R1*). Pathogenic variants in either gene lead to PI3Kδ hyperactivity.[5,6] This hyperactivity disrupts the development and function of lymphocytes and leads to decreased levels of naive B cells and increased levels of transitional B cells, senescent T cells, and follicular T_H cells, among other abnormalities.[2,3,7,8]

These aberrant lymphocyte populations often lead to simultaneous immunodeficiency and dysregulation, which present clinically as recurrent sinopulmonary infections, herpesvirus viremia, autoimmunity, enteropathy, or lymphoproliferation such as chronic lymphadenopathy, hepatosplenomegaly, and nodular lymphoid hyperplasia.[2,7,9,10] Patients also have an increased risk of lymphoma. The exact mechanism of malignancy is unknown, but elevated follicular T_H cells, reduced immunosurveillance, antigen- or EBV-driven pathway overactivation, or a combination of these may contribute.[11] APDS is progressive and can lead to end-organ damage, including hearing loss, bronchiectasis, and liver disease.[12]

Current management for APDS includes immunoglobulin replacement therapy (IRT), antimicrobial prophylaxis,

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Abbreviations used
AE: Adverse event
ALT: Alanine aminotransferase
APDS: Activated phosphoinositide 3-kinase delta syndrome
AST: Aspartate aminotransferase
CMV: Cytomegalovirus
COVID-19: Coronavirus disease 2019
CT: Computed tomography
DFT: Dose-finding trial
ED: Extension day
GI: Gastrointestinal
HSCT: Hematopoietic stem cell transplantation
IRT: Immunoglobulin replacement therapy
MRI: Magnetic resonance imaging
OLE: Open-label extension
PI3Kδ: Phosphoinositide 3-kinase delta
RCT: Randomized controlled trial (randomized, placebo-controlled, triple-blinded, phase 3 study)
SAE: Serious adverse event
TRAE: Treatment-related adverse event

symptom management, and hematopoietic stem cell transplantation (HSCT). Successful HSCT can resolve clinical symptoms of APDS, but this patient population has a high risk of engraftment failure and requires unplanned donor cell infusions. Adverse outcomes are frequent and serious and include multiple transplants, graft-versus-host disease, organ toxicity, severe infectious complications, and mortality. In addition, HSCT may not correct possible nonimmunologic manifestations of hyperactive PI3Kδ such as renal complications.[9,10,13-16] Lymphoma and transplant complications are common causes of death in patients with APDS.[9,13,15,17]

PI3Kδ inhibition is a promising therapeutic option for patients with APDS because it targets the specific pathway responsible for the disease.[5] Thus far, PI3K inhibitors targeting the δ, α, and/or γ isoforms are approved to treat hematological malignancies.[18] However, treatment with such inhibitors can cause serious adverse events (SAEs), including severe cutaneous reactions, hyperglycemia (on-target effect of PI3Kα inhibition),[19] severe neutropenia, increased risk of infections, pneumonitis, pneumonia, liver toxicities, and severe gastrointestinal (GI) AEs such as intestinal perforation and colitis.[20-25] Clinical trials of other PI3Kδ inhibitors such as nemiralisib and seletalisib for the treatment of APDS have been discontinued because of demonstrated lack of efficacy and probable lack of efficacy or AE severity, respectively.[26-28]

Previously published studies of leniolisib for the treatment of patients with APDS demonstrated rapid normalization of the PI3Kδ signaling pathway, resulting in reduction in lymphoproliferation accompanied by improved immune-cell subsets.[29,30] Leniolisib significantly reduced lymph node size and increased naive B-cell populations in patients enrolled in our recently published randomized controlled trial (RCT; a randomized, placebo-controlled, triple-blinded, phase 3 study).[30] Leniolisib restored the development of lymphocytes, reflected by reduced transitional B cells, reduced CXCL13, reduced $CD8^+$ senescent T cells, and normalized serum IgM levels. We now describe an open-label extension (OLE) study that also shows durable restoration of lymphocyte function.

METHODS

Patients

Patients who participated in the dose-finding trial (DFT) or the RCT (NCT02435173), or were previously treated with other PI3Kδ inhibitors, were eligible to enroll. Patients who were not previously enrolled in the DFT or RCT but met the same criteria established for the trials were allowed to enroll in the OLE study. Male and female patients were 12 to 75 years old, weighed 45 kg or more, and had pathogenic variants in *PIK3CD* or *PIK3R1,* with clinical manifestations of APDS.[29,30] Six patients were enrolled from the DFT and 29 patients were enrolled from the RCT. Of the 29 patients enrolled from the RCT, 20 were previously exposed to leniolisib and 9 received placebo. Two patients were newly enrolled to the OLE study and had historical PI3Kδ inhibitor exposure, but no previous leniolisib exposure (see Fig E1 in this article's Online Repository at www.jacionline.org).

Trial design and treatment

This planned interim analysis of an ongoing multinational, single-arm, open-label study assessed the long-term use of leniolisib for the treatment of patients with APDS. Six patients enrolled in the DFT and completed it by 2016. From the DFT, 5 patients entered the OLE study in 2016 and 1 in 2018 (see Fig E2 in this article's Online Repository at www.jacionline.org). The interim analysis was thus planned to coincide with 5 years of leniolisib exposure for this initial cohort. The RCT started in 2017, with rolling admission until May 2021, and was completed on August 16, 2021. After completing the 12-week trial, 29 of the 31 patients entered the OLE study. Two additional patients with APDS with previous exposure to a different PI3Kδ inhibitor entered the OLE in 2019 and 2020, for a total of 37 patients enrolled in the OLE study.

During the OLE study, all patients received 70 mg of leniolisib orally twice daily and will be followed for up to 7 years. The data cutoff date for this interim analysis was December 13, 2021, at which time patients received 70 mg of leniolisib orally twice daily for a median of 102 weeks (Table I). Five patients had treatment interruptions, 1 of which was immediately followed by a grade 5 AE, which is detailed in this article's Online Repository at www.jacionline.org. Twenty-one patients had assessment or procedure deviations due to coronavirus disease 2019 (COVID-19), and missing data point values is a limitation of this interim analysis.

End points and assessments

The primary end points of the OLE study are all safety parameters, including reported AEs and data collected via routine physical examinations, vital signs, hematologic parameters, blood chemistry, and urinalysis. Secondary end points include health-related quality-of-life assessment via patient answers to the 36-Item Short Form Survey, inflammatory markers, steady-state trough concentration of leniolisib, and frequency of infections. Safety parameters were assessed at every scheduled visit throughout the entirety of the study.

Exploratory outcomes assessed were hallmarks of the disease including reduction of lymphoproliferation as assessed via changes in spleen volume and lymph node size (determined through magnetic resonance imaging [MRI] or computed tomography [CT]) and B- and T-cell immunophenotyping. Other

TABLE I. Patient characteristics and exposure to study treatment

Characteristics	Previous leniolisib exposure (n = 26)	No previous leniolisib exposure (n = 11)	Total in OLE study (N = 37)
Age (y), median (range)	20.5 (12-55)	18.0 (14-49)	20 (12-55)
Sex: male/female (%)	57.7/42.3	54.5/45.5	56.8/43.2
Weight (kg), median (range)	65.5 (46.9-93.7)	71.20 (50.0-88.0)	67.10 (46.9-93.7)
Baseline IRT, n (%)	20 (76.9)	6 (54.5)	27 (73)
Leniolisib exposure, n (%)			
12-24 wk	4 (15.4)	2 (18.2)	6 (16.2)
26-36 wk	1 (3.8)	0 (0)	1 (2.7)
48-60 wk	2 (7.7)	3 (27.3)	5 (13.5)
72-84 wk	4 (15.4)	1 (9.1)	5 (13.5)
96-108 wk	3 (11.5)	3 (27.3)	6 (16.2)
108-156 wk	5 (19.2)	2 (18.2)	7 (18.9)
156-208 wk	2 (7.7)	0 (0)	2 (5.4)
208-260 wk	2 (7.7)	0 (0)	2 (5.4)
≥260 wk	3 (11.5)	0 (0)	3 (8.1)

assessments included cytokine and chemokine profiles, serum IgM levels, and cytomegalovirus (CMV) and EBV viremia. CMV and EBV viral loads were quantified from blood via PCR to determine the DNA copies per milliliter for each patient. Changes in regimen of other medications and IRT were assessed and reported from individual patient's clinical files. Efficacy readouts were assessed up until extension day (ED) 252. Assessments were descriptive in nature because this is a single-arm study and are reported as readouts over time. Imaging used to determine lymphoproliferation was done at either ED 168 or ED 252 and images were obtained only for patients previously enrolled in either the DFT or the RCT. Two patients not previously enrolled in the DFT or the RCT did not have available baseline images. MRI or CT scans used to assess lymphoproliferation were further stratified by previous exposure to leniolisib.

Trial oversight

Novartis Pharmaceuticals Corporation, in collaboration with investigators, designed the study. Novartis is overseeing the study's conduct and analyzing data, including statistical analysis, with Pharming Group NV. Data for the analysis are gathered locally, and protocol-defined laboratory samples and imaging are processed centrally. The trial is being conducted in accordance with the code of ethics of the World Medical Association (Declaration of Helsinki). The study population is representative (eg, sex, age, race, and ethnicity) of the population of patients with APDS at large. Independent ethics committees or institutional review boards at each center approved the protocol. Patients or their guardians provided written informed consent and assent. An independent monitoring committee is monitoring safety, data integrity, and protocol compliance.

RESULTS

Primary end-point analysis of safety parameters

The primary end point of the OLE study is safety of leniolisib treatment in patients with APDS. AEs were reported in 32 of the 37 patients enrolled in the extension study, with 5 patients reporting treatment-related AEs (TRAEs) and 6 patients reporting SAEs, which were determined not to be related to study drug treatment (Fig 1, *A*). Most AEs were of grades 1 and 2, and 10 patients experienced grade 3 AEs.

There were no grade 4 AEs, and 1 patient with significant baseline comorbidities (cardiomyopathy, tachycardia, recurrent pneumonia, necrotizing lymphadenitis, disseminated *Mycoplasma orale* infection, bronchiectasis, pancytopenia, liver disease, and peripheral edema) experienced cardiac arrest, resulting in death at ED 879 (Fig 1, *B*). This was determined not to be related to study drug treatment.

The most common AEs reported were upper respiratory tract infection (9 patients), headache (6 patients), pyrexia (6 patients), otitis externa (5 patients), COVID-19 (5 patients), and increased weight (5 patients) (Table II). GI AEs were reported in 15 patients, 10 of whom had a history of GI disease; 85.3% of these GI AEs were considered grade 2 or lower. Increased weight (3 patients), arthralgia (1 patient), hyperglycemia (1 patient), and decreased neutrophil count (1 patient) were reported as treatment-related (Table III). Notably, the neutropenia resolved during the study. Furthermore, the patient who experienced hyperglycemia had a history of uncontrolled diabetes. There were no GI TRAEs, and none of the TRAEs led to treatment interruptions.

SAEs are detailed in Table III. Most SAEs were reported in only 1 patient and included vomiting, sinusitis, anal fissure, and colitis. Abdominal pain and increased alanine aminotransferase (ALT) levels were each reported as SAEs in 2 patients. Increases in ALT levels returned to normal after approximately 2 weeks. The rate of SAEs was numerically higher in the group with no previous exposure to leniolisib than in the group with previous leniolisib treatment (see Table E1 in this article's Online Repository at www.jacionline.org). None of the SAEs reported were related to the study treatment.

Values of the assessed safety laboratory markers, vital signs, and electrocardiogram remained stable and within normal limits over time with leniolisib treatment. There were no notable distinctions in values between patients with previous leniolisib treatment and patients with no previous leniolisib exposure (data not shown).

Secondary and exploratory outcomes

Lymphoproliferation outcomes. All patients enrolled in the OLE study experienced a 62.7% reduction in mean index lymph node size and a 37.6% reduction in mean spleen volume

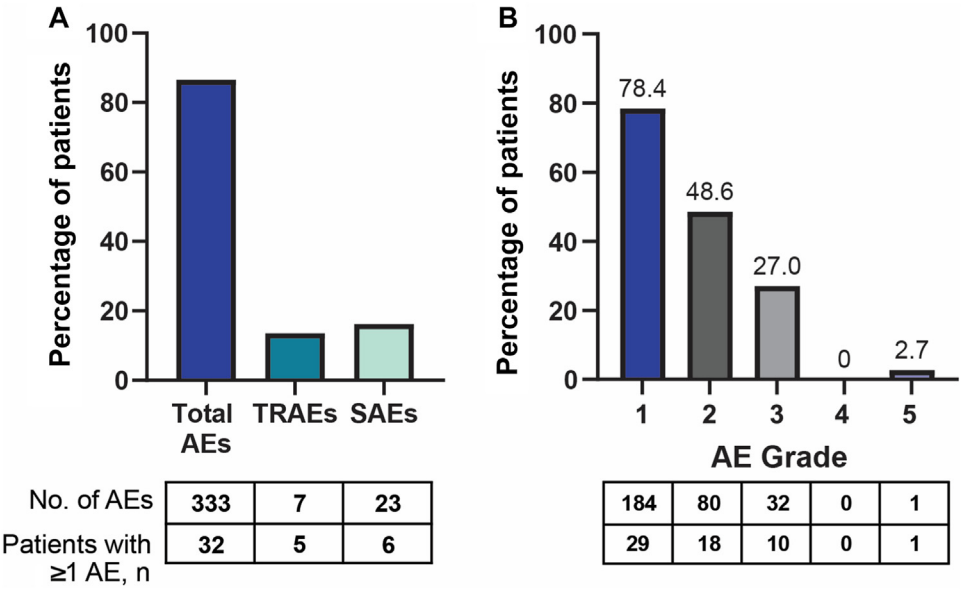

FIG 1. AEs and AE grades. **A**, Total percentage of patients with all reported AEs, TRAEs, and SAEs. **B**, Percentage of patients stratified by grades of AEs.

while on leniolisib from the screening to the first OLE assessment. Patients with previous exposure to leniolisib during the DFT or the RCT had a continued reduction in lymphoproliferation in the OLE study, with 14 and 15 patients experiencing further reductions in index lymph node size (n = 19) and spleen volume (n = 20), respectively. During the RCT, some patients placed on placebo (n = 9) experienced increases in index lymph node size (n = 2) and spleen volume (n = 6). Of these patients with available lymph node size (n = 7) and spleen volume (n = 6) measurements, during the OLE all demonstrated durable reduction in lymphoproliferation (Fig 2, *A-D*).

Finally, 3 patients in the original DFT had a history of lymphoma[29]; none had relapses or new lymphomas up to the interim analysis cutoff.

Immune outcomes. The mean percentage of naive B cells and transitional B cells came within normal limits by ED 84 and remained in range through the last measurement (Fig 3, *A* and *B*). The mean percentage of mature B cells increased from ED 1 to ED 252 (Fig 3, *C*). Elevated plasmablasts were reduced to normal by ED 168 and this was sustained (Fig 3, *D*). Switched memory B cells normalized by ED 168 (Fig 3, *E*). We assessed CXCL13, which recruits B and T cells to the lymph node or to form lymphoid follicles.[31] Serum levels of CXCL13 are often increased in APDS. Patients had elevated CXCL13 levels at ED 1 (273.8 pg/mL), which were reduced to be within normal limits to 107.0 pg/mL by ED 252 (Fig 3, *F*). Patients who had high IgM levels at the start of the extension period (n = 10) saw a reduction in IgM levels, with 3 patients reaching normal levels by ED 84, which was maintained through ED 252 (Fig 3, *G*).

$CD8^+$ senescent T cells were maintained within normal limits throughout and $CD4^+$ senescent T cells were reduced to normal by ED 168 but were just out of normal range at ED 252 (Fig 4, *A*). PD-1^+ $CD4^+$ and $CD8^+$ cells were within normal range throughout the study (Fig 4, *B*). At ED 1, the inverted $CD4^+$:$CD8^+$ T-cell ratio was 0.82; this normalized to 1.32 by ED 252 (Fig 4, *C*). Central memory $CD8^+$ T cells remained stable in the normal range, whereas central memory $CD4^+$ and effector memory T cells were elevated but stable (Fig 4, *E* and *F*). $CD4^+$ effector memory T cells re-expressing CD45RA oscillated between in range and just out of range throughout the study, and $CD8^+$ T-cell levels reduced and were just at the maximum limit of normal (Fig 4, *G*). Numerical changes in lymphocytes between ED 1 and ED 252 are provided in Table E2 (in the Online Repository available at www.jacionline.org).

We assessed levels of IgA, IgE, and distinct subclasses of IgG during the OLE study. Ten patients had low levels of IgE at the start of the OLE study; 4 of them had increased levels by ED 252 (see Fig E3, *A*, in this article's Online Repository at www.jacionline.org). IgA levels remained stable and within normal range for most patients throughout ED 252 (Fig E3, *B*). Four patients had increased IgA levels at distinct time points that coincided with infections; however, these values were still considered within normal limits. All patients had IgG levels within normal limits throughout, and there were no notable changes observed in IgG subclasses; however, 27 of 37 patients were on IRT concomitant to leniolisib for much of the study (Fig E3, *C-G*). There were no observable differences between patients with and without previous leniolisib exposure.

Representative lymphocyte parameters of individual results. To assess the effective transition onto leniolisib for patients who were previously placed on placebo during the RCT, individual B- and T-cell parameters for naive B cells, plasmablasts, CXCL13, and senescent T cells are illustrated in Fig E4 (in the Online Repository available at www.jacionline.org). The overall observed trends were similar to the trends observed in the graphs of the mean values. Importantly, most patients who were previously on placebo demonstrated a normalization as of ED 84 or ED 168 of the OLE study and most patients reached normal range within measured lymphocyte subsets and other immune parameters by ED 252.

Infection-related outcomes and IRT use *post hoc* analyses. Two *post hoc* analyses were performed to assess clinically relevant changes in infection rates and IRT usage. Of the patients enrolled in the extension period, 27 were receiving IRT

TABLE II. Most common AEs reported in >5% of the whole OLE cohort according to grade severity

Most common AEs reported by preferred term	Incidence of AEs, n (%)	
	Grade 1-2	Grade ≥3
Abdominal pain	2 (5.4)	2 (5.4)
Upper abdominal pain	2 (5.4)	0
Diarrhea	4 (10.8)	0
Gastroesophageal reflux disease	3 (8.1)	0
Noncardiac chest pain	2 (5.4)	0
Pyrexia	5 (13.5)	1 (2.7)
COVID-19	5 (13.5)	0
Folliculitis	2 (5.4)	0
Gastroenteritis	2 (5.4)	0
Herpes zoster	2 (5.4)	0
Influenza	2 (5.4)	0
Lyme disease	2 (5.4)	0
Nasopharyngitis	2 (5.4)	0
Oral herpes	2 (5.4)	0
Otitis externa	5 (13.5)	0
Otitis media	3 (8.1)	0
Acute otitis media	2 (5.4)	0
Pharyngitis	4 (10.8)	0
Respiratory tract infection*	4 (10.8)	0
Rhinitis	3 (8.1)	0
Sinusitis	3 (8.1)	1 (2.7)
Upper respiratory tract infection	9 (24.3)	0
Ligament sprain	2 (5.4)	0
Increased ALT	0	3 (8.1)
Increased AST	0	2 (5.4)
Weight increase	2 (5.4)	3 (8.1)
Obesity	0	2 (5.4)
Arthralgia	3 (8.1)	0
Back pain	2 (5.4)	0
Myalgia	2 (5.4)	0
Headache	6 (16.2)	0
Asthma	2 (5.4)	0
Epistaxis	2 (5.4)	0
Oropharyngeal pain	2 (5.4)	0
Alopecia	2 (5.4)	0
Keratosis pilaris	2 (5.4)	0
Rash	2 (5.4)	0
Seborrheic dermatitis	3 (8.1)	0

*Three of the 4 patients with respiratory tract infection AEs were also reported to have upper respiratory tract infections and it is possible they are counted twice.

TABLE III. TRAEs and SAEs reported

AEs reported by preferred term	Incidence of AEs, n (%)
TRAEs	
Decreased neutrophil count	1 (2.7)
Weight increase	3 (8.1)
Hyperglycemia	1 (2.7)
Arthralgia	1 (2.7)
SAEs	
Cardiac arrest	1 (2.7)
Abdominal pain	2 (5.4)
Anal fissure	1 (2.7)
Colitis	1 (2.7)
Hematochezia	1 (2.7)
Vomiting	1 (2.7)
Facial pain	1 (2.7)
Pyrexia	1 (2.7)
Soft tissue abscess	1 (2.7)
Acute sinusitis	1 (2.7)
Parotitis	1 (2.7)
Periorbital cellulitis	1 (2.7)
Pneumonia	1 (2.7)
Sinusitis	1 (2.7)
Increased ALT	2 (5.4)
Increased AST	1 (2.7)
Dehydration	1 (2.7)
Hypocalcemia	1 (2.7)
Reactive arthritis	1 (2.7)
Aspiration	1 (2.7)
Orthostatic hypotension	1 (2.7)

concomitant to leniolisib at the time of screening. By the cutoff date, 37% of patients who were receiving IRT at the start of the study had decreased use of IRT. Specifically, 1 patient had a 30% reduction, 3 patients had a 50% reduction, and 6 patients had stopped the use of IRT, although 1 patient had a one-time IRT after discontinuing use. Four patients who discontinued IRT had been IRT-free for 1 to 2.5 years as of the data cutoff date.

One-hundred eight infections were experienced by 26 of the 37 patients. There was a statistically significant decrease of −0.351 ($P = .0040$) in annualized infection rates with each additional year of leniolisib treatment. Patients on leniolisib were also observed to have a reduction in the number of infection days (data not shown). This decrease in annualized infection rate was accompanied by no appreciable increase in antibiotic use.

Hematological parameters. At the start of the OLE study, patients with and without previous leniolisib exposure had cytopenias. Nine patients (5 female and 4 male patients) had anemia, which resolved in 5 patients (55%) by the data cutoff date (Fig 5, *A* and *B*). Another patient had improved hemoglobin levels, but not to normal limits (Fig 5, *B*). Of the 6 patients who began the OLE study with thrombocytopenia, 3 resolved, 2 improved, and 1 remained stable (Fig 5, *C*). Two of the 5 patients who had lymphopenia also improved (Fig 5, *D*). Finally, neutropenia resolved for the 3 patients who began the OLE neutropenic (Fig 5, *E*).

CMV and EBV viremia. At the initial screening for the OLE study, 12 patients had positive test results for either EBV or CMV; there was no increase in viral load for EBV or CMV reported in any of these patients. The greatest values detected were 156 DNA copies/mL for CMV and 488 DNA copies/mL for EBV (data not shown).

Inflammatory markers. The mean values of high-sensitivity C-reactive protein and lactose dehydrogenase stayed within normal limits (see Fig E5, *A* and *B*, in this article's Online Repository at www.jacionline.org). Individual fluctuations out of normal limits corresponded to infections and resolved accordingly.

Other inflammatory markers were largely stable and unremarkable, although there were relevant reductions to markers that were out of range (Fig E5, *C-G*). One patient experienced increased levels of TNF and IFN-γ–induced protein 10 at all 4 time points (ED 1, ED 84, ED 168, and ED 252); concomitantly, at ED 1 and ED 252, the patient had pneumonia and rhinitis, respectively. There were no notable differences between patients with and without previous leniolisib exposure.

Health-related quality of life. Health-related quality of life was assessed through patient- and physician-reported outcomes. Overall, the physical component scores and the mental component scores were maintained through ED 728 (see Fig E6, *A* and *B*,

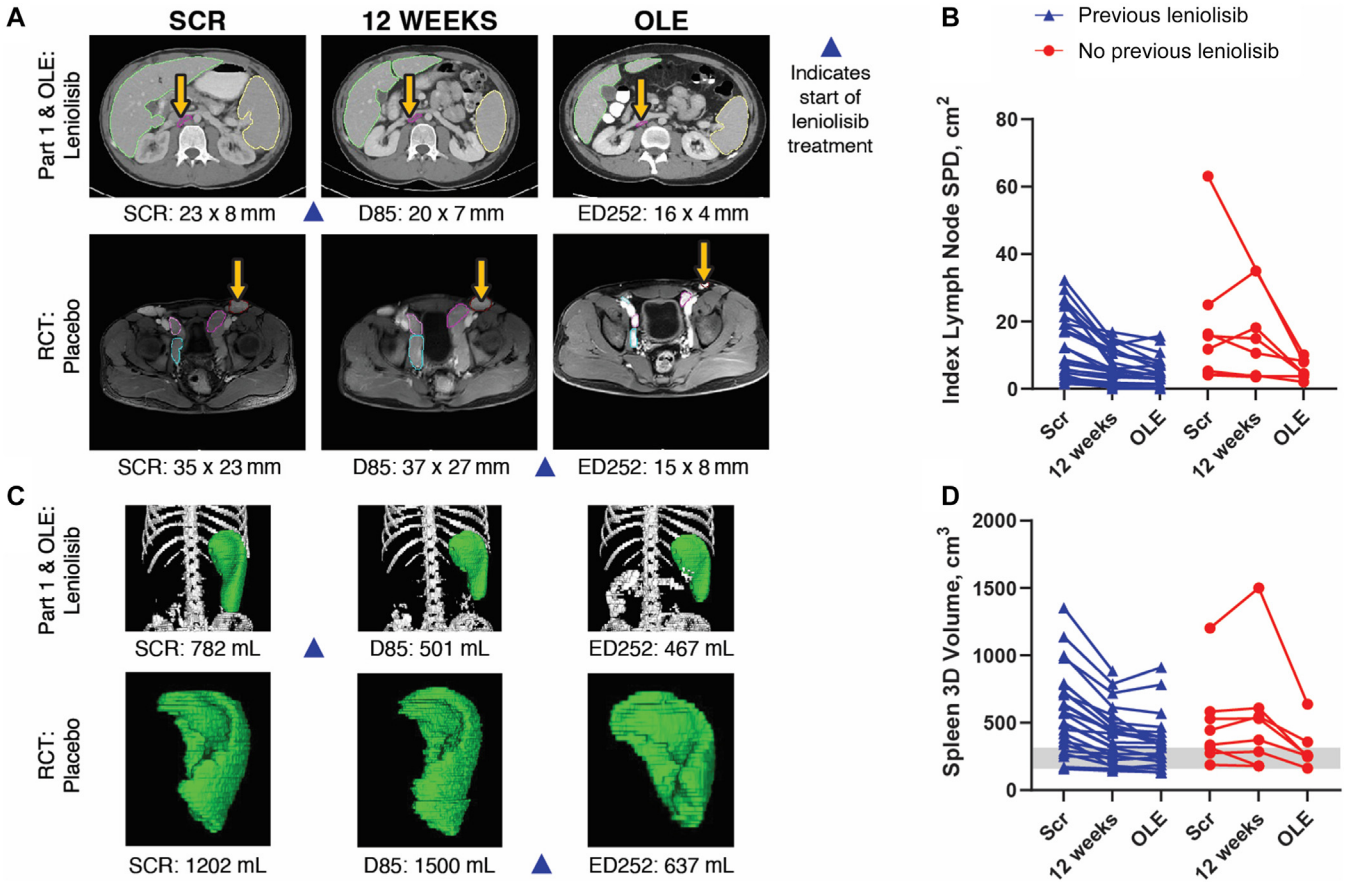

FIG 2. Lymphoproliferation outcomes. **A**, Representative radiographic renderings of lymph node diameters from patients at screening (Scr), end of the DFT or RCT (12 weeks), and readout at either ED 168 or ED 252 of the OLE study. **B**, Individual values of untransformed sum of product diameters (SPD) of index lymph nodes for Scr (n = 24; n = 8), 12 weeks (n = 24; n = 7), and OLE study (n = 19; n = 7) (previous leniolisib exposure; no previous leniolisib exposure). **C**, Representative radiographic renderings of spleen volumes from patients at Scr, 12 weeks, and OLE. **D**, Individual spleen volumes for Scr (n = 25; n = 8), 12 weeks (n = 25; n = 8), and OLE study (n = 20; n = 6) (previous leniolisib exposure; no previous leniolisib exposure). Gray-shaded box indicates normal range.

in this article's Online Repository at www.jacionline.org). In addition, the norm-based score of the general health domain was improved by 7.8 points by ED 728 as compared with the baseline values of individuals who had values at both time points (Fig E6, *C*). This change can be considered clinically meaningful in patients with primary immunodeficiencies.[32] The overall activity impairment due to health in both the patient and the physician general assessment scores was maintained through ED 728 (Fig E6, *D-F*).

DISCUSSION

This interim analysis of the OLE study demonstrates that long-term use of leniolisib for the treatment of patients with APDS was well tolerated with sustained improvements in disease markers. This study represents one of the longest follow-ups in targeted treatment studies in the field of inborn errors of immunity,[33,34] and certainly the first long-term study of a PI3Kδ inhibitor in patients with APDS, with more than 5 years of leniolisib exposure in 3 patients.

Leniolisib binds the p110δ lipid kinase subunit and inhibits the disease-causing enzyme complex, PI3Kδ, making leniolisib a precision therapeutic option for patients with APDS.[7,35] We surmise that the precise match of mechanisms of disease and therapeutic intervention contributes to the tolerability, in addition to appropriate dosing and other unique leniolisib qualities. Most of the reported AEs were classified as grade 1 (78.4%), with upper respiratory tract infection, headache, and pyrexia being the most commonly reported AEs. None of the SAEs were determined to be treatment-related by the investigator. Importantly, most of the GI AEs were grade 1 or 2 and were unrelated to the study treatment. This is a critical finding, because other PI3Kδ inhibitors used to treat patients with hematologic malignancies have caused severe GI AEs that typically manifest after 6 months of treatment, although mild GI AEs presented as early as 8 weeks.[20,36] In addition, other PI3Kδ inhibitors have reported other significant AEs, such as liver toxicities (including grade 3 or higher transaminitis), pneumonitis, intestinal perforation, grade 3 or higher cytopenias, and severe cutaneous reactions,[21-25] most of which were not observed with long-term leniolisib exposure. The transaminitis and neutropenia that were seen in the leniolisib study were

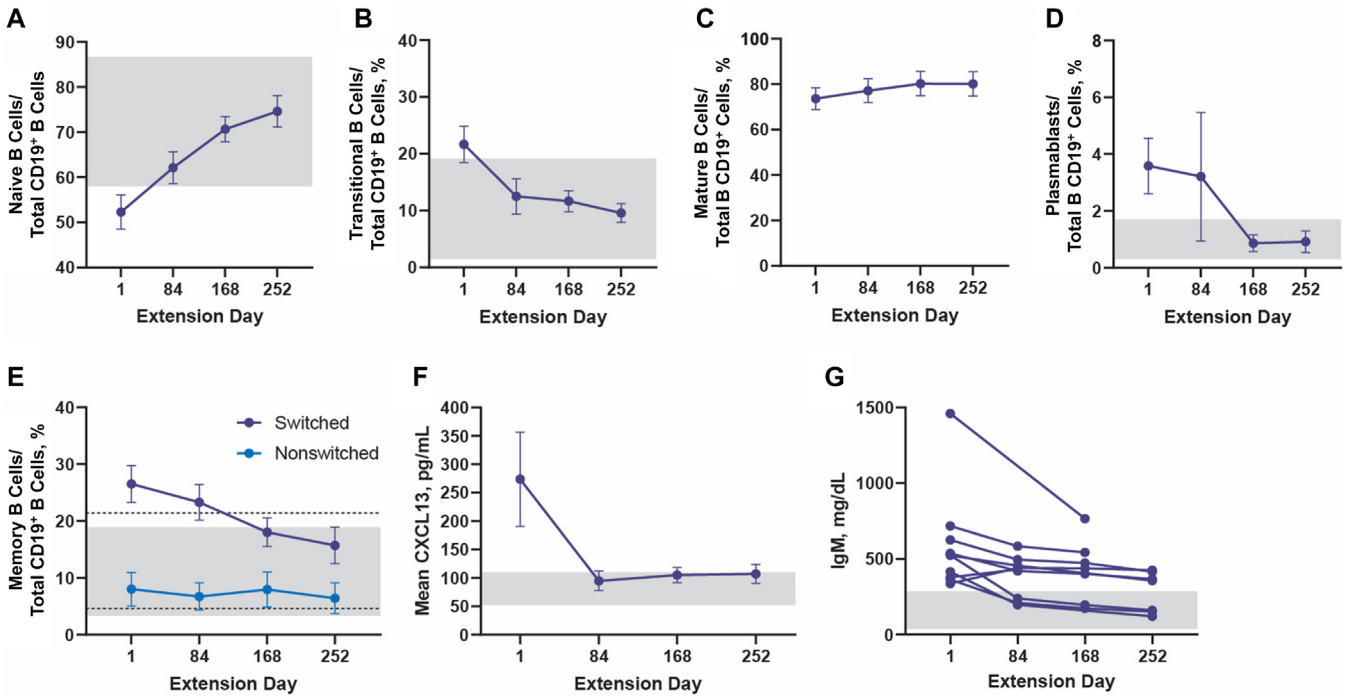

FIG 3. Changes in B-cell and immune parameters. **A**, Mean naive B-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252: 34, 34, 28, 24). **B**, Mean transitional B-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252: 34, 34, 28, 24). **C**, Mean mature B-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252: 34, 34, 28, 24). **D**, Mean plasmablast ($CD19^+CD27^+CD38^{++}$) percentages over time (n values for ED 1, ED 84, ED 168, and ED 252: 34, 34, 28, 23). **E**, Mean switched ($CD19^+CD27^+IgD^-$) and nonswitched ($CD19^+CD27^+IgD^+$) memory B-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252 for each group: switched memory B cells, 34, 34, 28, 24; nonswitched memory B cells, 34, 34, 28, 24). **F**, Mean values of circulating CXCL13 for ED 1 (n = 31), 84 (n = 29), 168 (n = 26), and 252 (n = 26). **G**, Individual IgM values over time (n values for ED 1, ED 84, ED 168, and ED 252: 10, 9, 7, 7). In Fig 3, *A* to *D*, and *F* and *G*, the gray-shaded boxes indicate normal range values for respective measurements. In Fig 3, *E*, the gray-shaded box indicates normal range for switched memory B cells, and the dashed lines indicate normal range for nonswitched memory B cells.

temporary. Grade 3 or higher AEs were reported in 54% and 48% of patients treated with idelalisib for non-Hodgkin lymphoma or chronic lymphocytic leukemia in phase 2 and phase 3 studies, respectively.[36] Here, we report fewer than 30% of patients with grade 3 or higher AEs after long-term leniolisib exposure (Fig 1). The differences in the chemical structure of leniolisib could account for the differences in liver, GI tract, and other organ toxicities seen with other PI3Kδ inhibitors.[35,37-39]

From the DFT through the OLE study, we see evidence of sustained disease modification spanning from the cellular pathway to lymphocyte subsets to clinical symptoms. In the DFT, we showed that leniolisib inhibits the hyperactive PI3Kδ signaling pathway, as measured by phosphorylated AKT.[29] During the RCT, leniolisib partially reversed immune dysregulation through significant reduction in lymphadenopathy and normalization of immune-cell subsets, especially naive B-cell populations and other immune parameters compared with the placebo arm.[30] Here, we show a significant reduction in the annualized rate of infections as well as days with infections. Furthermore, all 6 patients who stopped IRT during the OLE study sustained normal IgG, IgE, and IgA levels, and any infections seen in these patients resolved in normal time frames, without increased antibiotic use. Thus, patients taken off IRT have functional B cells and can make antibodies, which controlled infections.

Furthermore, a sustained reduction in lymphoproliferation, assessed by lymph node size and spleen volume, was observed. Most patients receiving placebo during the RCT either remained stable or had an increase in both spleen volume and lymph node size by day 85 of the RCT; however, once the patients on placebo were switched to leniolisib treatment, this was reversed and lymphoproliferation reduced further compared with baseline measurements by the first analysis of the OLE study. Patients who had previous leniolisib treatment in either the DFT or the RCT maintained a progressive reduction in both spleen and lymph node volumes through the first OLE analysis. This observation demonstrates a prompt and durable reduction of lymphoproliferation.

It was not an exclusion criterion, but patients with clinically significant autoimmune cytopenias were not enrolled in this trial because they would have been potentially intolerant of the immunosuppressive agent washout period or of being placed on placebo during the preceding RCT. As such, the current patient population in the OLE study may not be representative of the range of cytopenia severities observed in the overall population of patients with APDS. Nevertheless, most of the patients with cytopenias at the start of the OLE study had improvement while on leniolisib as of the data cutoff date.

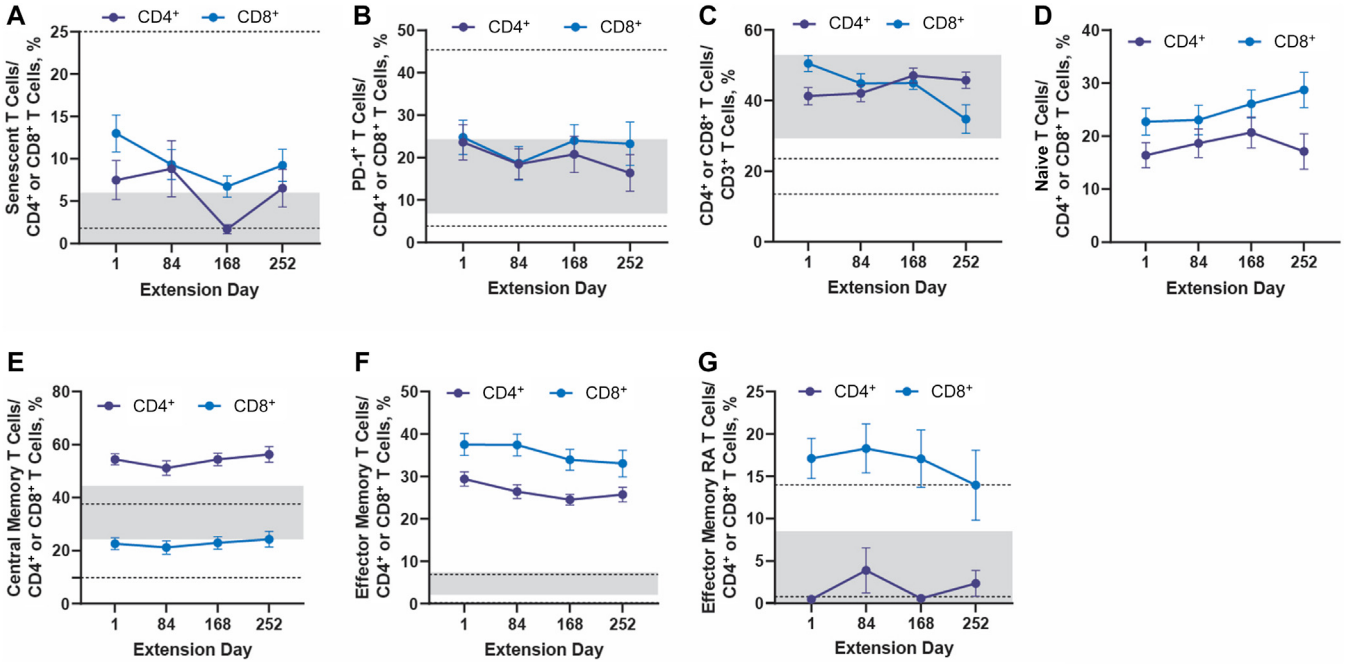

FIG 4. T-cell parameters. **A**, Mean senescent T-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252 for each group: $CD4^+$, 31, 32, 28, 24; $CD8^+$, 30, 32, 28, 21). **B**, Mean $CD4^+$ and $CD8^+$ $PD\text{-}1^+$ T-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252 for each group: $CD4^+$, 32, 33, 28, 24; $CD8^+$, 32, 32, 28, 20). **C**, Mean $CD4^+$ and $CD8^+$ T-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252 for each group: $CD4^+$, 32, 33, 28, 24; $CD8^+$, 32, 33, 28, 24). **D**, Mean naive ($CD45RA^+$ $CD62L^+$) $CD4^+$ and $CD8^+$ T-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252 for each group: $CD4^+$, 31, 33, 28, 24; $CD8^+$, 32, 32, 28, 21). **E**, Mean central memory ($CD45RO^+$ $CD62L^+$) $CD4^+$ and $CD8^+$ T-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252 for each group: $CD4^+$, 32, 33, 28, 24; $CD8^+$, 32, 32, 28, 21). **F** and **G**, Mean effector memory and effector memory cells re-expressing CD45RA ($CD45RA^+$ $CD62L^+$) $CD4^+$ and $CD8^+$ T-cell percentages over time (n values for ED 1, ED 84, ED 168, and ED 252 for each group: effector memory, $CD4^+$, 32, 33, 28, 24; $CD8^+$, 32, 32, 28, 21; effector memory RA, $CD4^+$, 31, 32, 28, 27; $CD8^+$, 32, 32, 28, 21). Gray-shaded boxes indicate normal ranges for $CD4^+$ cells, and dashed lines indicate normal range for $CD8^+$ cells.

PI3Kδ hyperactivity in APDS results in clinically relevant alterations in B- and T-cell populations, with reduced levels of naive B cells, increased levels of transitional B cells, and increased markers of T-cell senescence and exhaustion.[4,8,9,12] During the OLE study, naive and transitional B-cell populations came within normal limits, whereas markers of T-cell senescence and exhaustion were reduced.

Overall, long-term exposure to leniolisib was well tolerated in patients with APDS and led to progressive improvement in the pathognomonic features of APDS. Here, we posit that leniolisib treatment may be considered disease-modifying, demonstrated by the improvement in immunodeficiency with a reduction in IRT use while also demonstrating a significant reduction in annualized infection rates.[1,9,30] The OLE study is ongoing; however, this interim analysis study highlights the long-term tolerability and clinical impact that leniolisib treatment may provide for patients with APDS.

DISCLOSURE STATEMENT

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Disclosure of potential conflict of interest: S. Webster is a consultant for Pharming Group NV. A. Šedivá is a consultant for Octapharma, Takeda, and Pharming NV. A. Shcherbina receives honoraria from and is a consultant for Octapharma, CSL Behring, and Novartis. E. Kulm is an employee of Leidos Biomedical Research, Inc. J. Körholz has received honorarium from Pharming Group NV. V. A. Dalm is a consultant for and/or receives honoraria from AstraZeneca, Kedrion, Takeda, CSL Behring, Pfizer, and Pharming Group NV; and receives research funding from Takeda. K. Radford is an employee of Novartis Pharma AG. A. Relan is an employee and stock option holder of Pharming Group NV. J. Bradt is an employee and stock option holder of Pharming Group NV; and holds individual stock in Neoclone. The rest of the authors declare that they have no relevant conflicts of interest.

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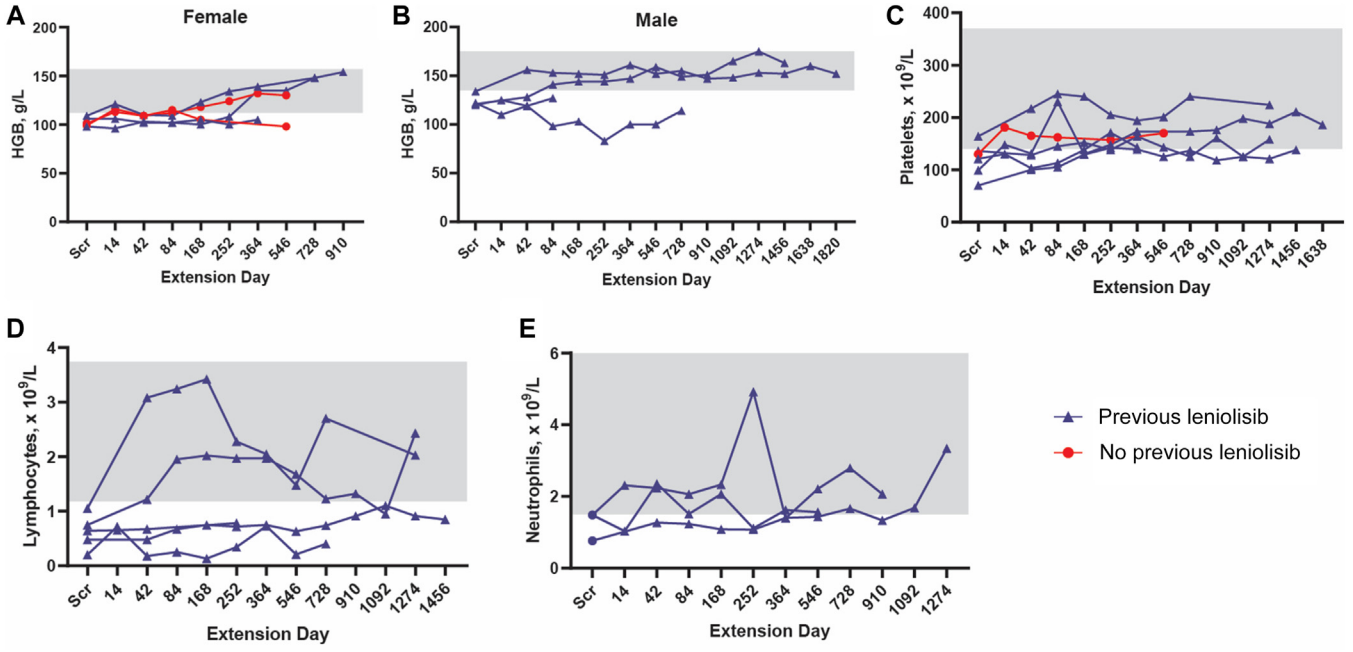

FIG 5. Changes in baseline cytopenias. **A**, Individual female patients' hemoglobin levels over time (n = 5). **B**, Individual male patients' hemoglobin levels over time (n = 4). **C**, Platelet levels over time for individual patients who started with low levels at screen time (n = 6). **D**, Lymphocyte values over time for individual patients (n = 5). **E**, Neutrophil levels over time for individual patients (n = 3). Gray-shaded boxes indicate normal range values for respective measurements.

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Clinical implications: Patients with APDS have limited treatment options that target the mechanism of disease, hyperactive PI3Kδ signaling. Here, we report long-term implications of leniolisib, a targeted orally bioavailable PI3Kδ inhibitor, in APDS.

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METHODS

Patients

The key inclusion criteria are as described in the main article. However, some additional exclusion criteria were followed. Patients were excluded if they met any of the following: withdrew consent from either the DFT or the RCT, had previous use of other investigational drugs within 30 days, had a history of hypersensitivity to any study treatments of similar chemical classes, or had a history of electrocardiogram abnormalities. Pregnant or lactating women were excluded, and women with childbearing potential were eligible only if highly effective contraceptive methods were used. Patients with a history of any surgical or medical condition that could potentially impair absorption, distribution, metabolism, or excretion of the study treatment were also excluded. Patients with a positive hepatitis B or C test result were not considered for this study.

Twenty-one patients had protocol deviations due to COVID-19 restrictions. Protocol deviations due to COVID-19 restrictions included assessment or procedure changes, changes in drug supply methods, visits done outside of the study site, and missed visits.

Baseline determination

For most assessments, values were obtained starting from day 1 of the OLE study. However, specifically for the cytopenias assessed at screen time or baseline, the timing of when these values were obtained differed slightly depending on the patient. Baseline values, from patients previously enrolled in either the DFT or the RCT (core study), with a study treatment gap of no longer than 6 weeks between the end of the core study and the start of the OLE study, were used from the core study. For patients who were not previously enrolled in the core study or who had a gap in study treatment longer than 6 weeks, baseline values were obtained at the start of the OLE study.

End points and assessments

AEs were reported through clinical assessments, nondirective interviewing of each patient, or as volunteered by the patient. Detected AEs were followed until resolution. Frequency of infections was reported similarly to AEs through nondirective interviews of each patient or as volunteered by the patient at each visit; no patient-reported outcome instrument was used to measure infections in between visits, relying on patient recall at each visit to recall past infections.

Lymphoproliferation was assessed via MRI or CT scans. The Cheson criteria were applied when selecting and measuring nodal and extranodal index lymph nodes.[E1] Index lymph node size and spleen volume were stratified by previous leniolisib exposure and no previous leniolisib exposure over time, starting from initial screening to the end of the RCT and the first readout of the OLE study.

B- and T-cell immunophenotyping were conducted via flow cytometry for key cellular markers of distinct cell types. Percentage of each B-cell type was calculated as compared with total $CD19^+$ B cells. Percentage of each T-cell type was calculated as compared with either total $CD4^+$ or total $CD8^+$ T cells. Normal ranges for percentages of B- and T-cell subsets included throughout were based on previously published values for patients from 11 years and into adulthood.[E2,E3]

Yearly rates of infections were assessed as a log-linear negative binomial model fit to the infection count data, including an offset for years spent in study, and the presence of baseline infection as a covariate.

Although the data were not shown, viral loads from both CMV and EBV were assessed via quantitative PCR and determined as DNA copy number in circulation for each patient. Serum immunoglobulin levels and several inflammatory markers were quantified from blood to determine systemic levels of each, including high-sensitivity C-reactive protein, lactate dehydrogenase, TNF, and IFN-γ–induced protein 10. Specific macrophage inflammatory markers were also assessed and included: macrophage-derived chemokine and macrophage inflammatory proteins 1β and 3α.

Serum immunoglobulin values were censored for certain patients because the values listed were out of physiological range. Censoring occurred for 9 patients for IgA and IgG, 8 patients for IgM, 5 patients for each IgG1 to IgG4, and 3 patients for IgE.

RESULTS

Treatment interruptions

Five patients had dose interruptions. Three of these patients had interruptions due to administration of steroids or other prohibited medication, and the fourth patient had a 10-day dose interruption due to increased ALT and AST, likely due to Tylenol toxicity. Two patients had 3 interruptions each. In 1 patient, all the interruptions were due to prednisone or methylprednisolone administration, and in the other patient, 2 interruptions were due to prednisone administration and a third interruption was due to colitis and consequent administration of a prohibited medication. One patient had a dose interruption due to elevated AST levels, which was immediately followed by a grade 5 AE. As described in the main article, this patient had significant comorbidities.

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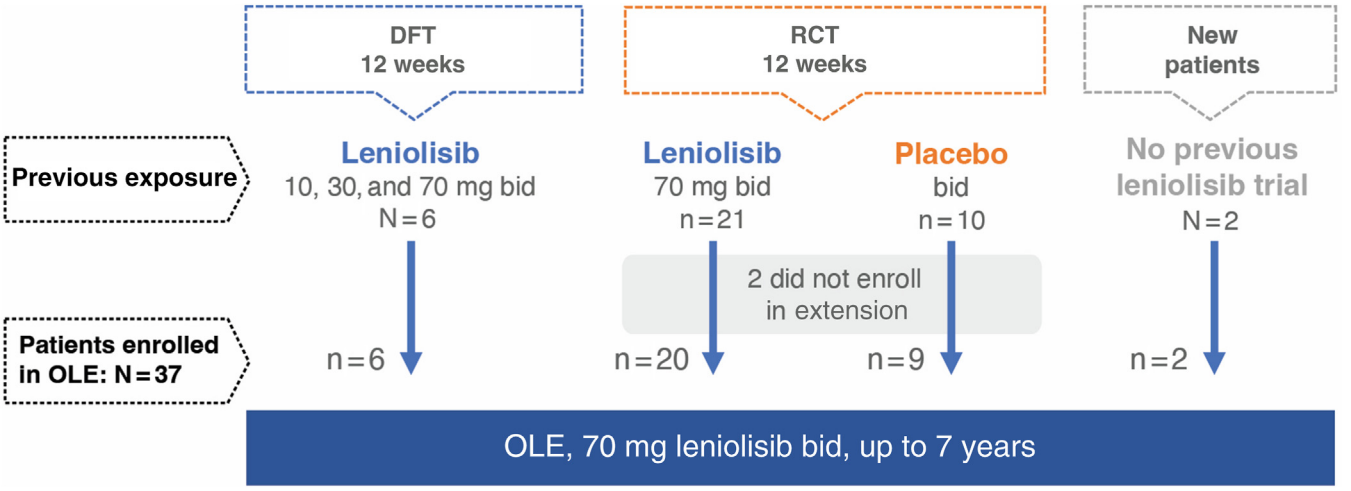

FIG E1. Study design and patient enrollment. Schematic of patients included from the DFT, the RCT, or the newly enrolled, and the specific previous exposure and dose. Of the 2 patients from the RCT who did not enroll in the OLE, 1 from the placebo arm clinically worsened leading to death while awaiting OLE initiation and implementation, while the other in the study drug arm did not wish to pursue long term leniolisib treatment. *bid,* Twice daily.

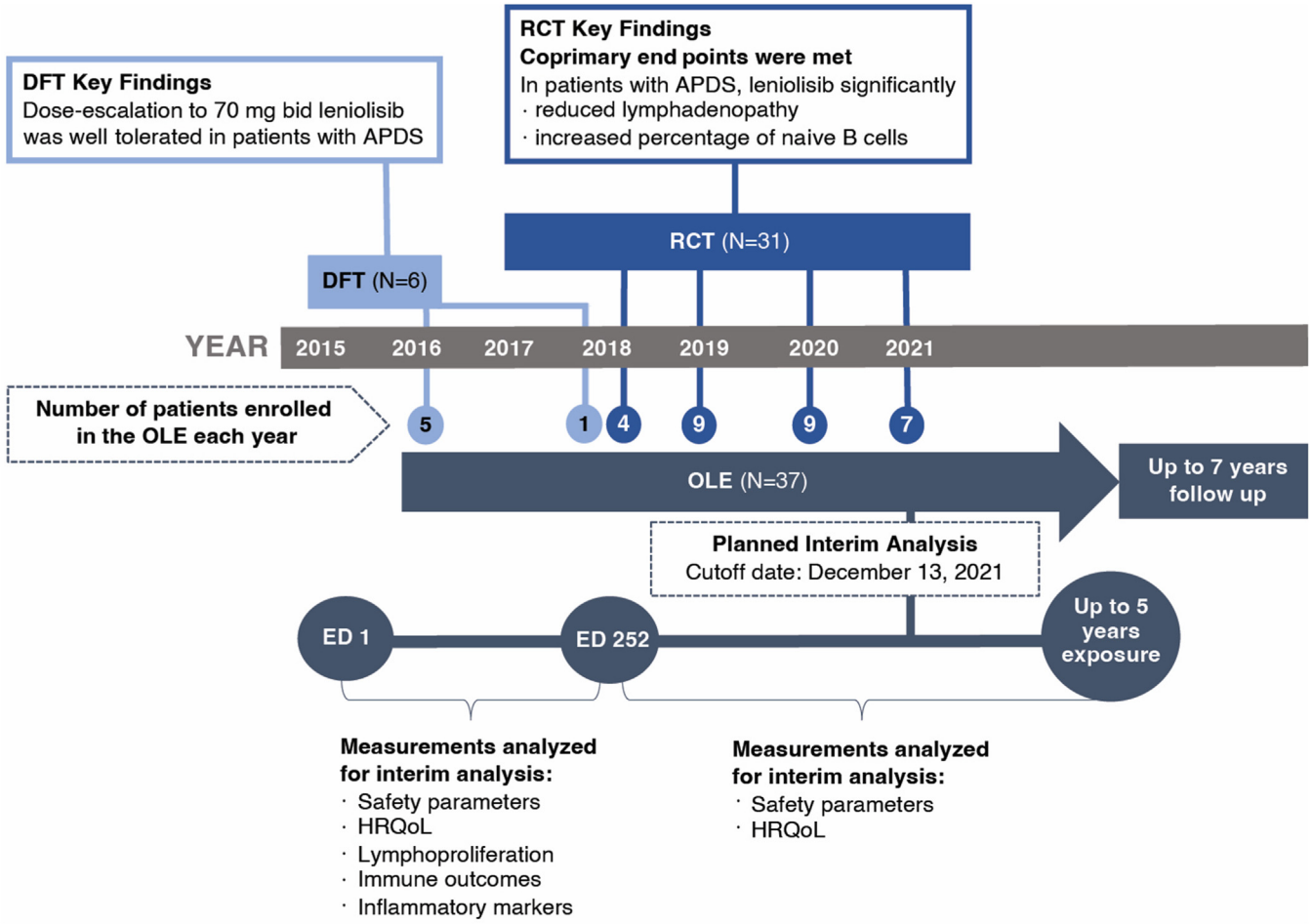

FIG E2. Timeline of leniolisib patient trials and enrollment into OLE study. Timelines for the DFT[E4] and the RCT[E5] and patient enrollment into the OLE study. *bid*, Twice daily; *HRQoL*, health-related quality of life.

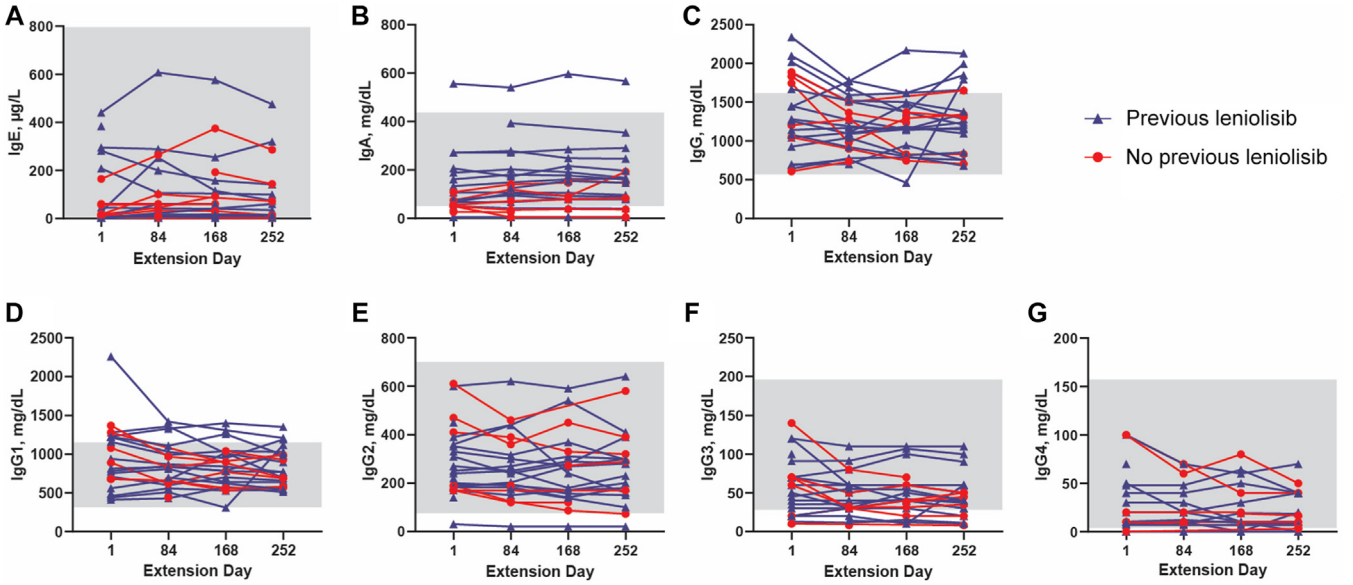

FIG E3. Immunoglobulin changes over time. **A**, Individual IgE levels over time for ED 1 (n = 27), ED 84 (n = 23), ED 168 (n = 20), and ED 252 (n = 20). **B**, Individual IgA levels over time for ED 1 (n = 21), ED 84 (n = 23), ED 168 (n = 21), and ED 252 (n = 22). **C**, Individual IgG levels over time for ED 1 (n = 22), ED 84 (n = 23), ED 168 (n = 21), and ED 252 (n = 22). **D**, Individual IgG1 levels over time for ED 1 (n = 24), ED 84 (n = 22), ED 168 (n = 20), and ED 252 (n = 22). **E**, Individual IgG2 levels over time for ED 1 (n = 24), ED 84 (n = 22), ED 168 (n = 20), and ED 252 (n = 22). **F**, Individual IgG3 levels over time for ED 1 (n = 24), ED 84 (n = 22), ED 168 (n = 20), and ED 252 (n = 22). **G**, Individual IgG4 levels over time for ED 1 (n = 24), ED 84 (n = 21), ED 168 (n = 20), and ED 252 (n = 21). Gray-shaded boxes indicate normal range values for respective measurements.

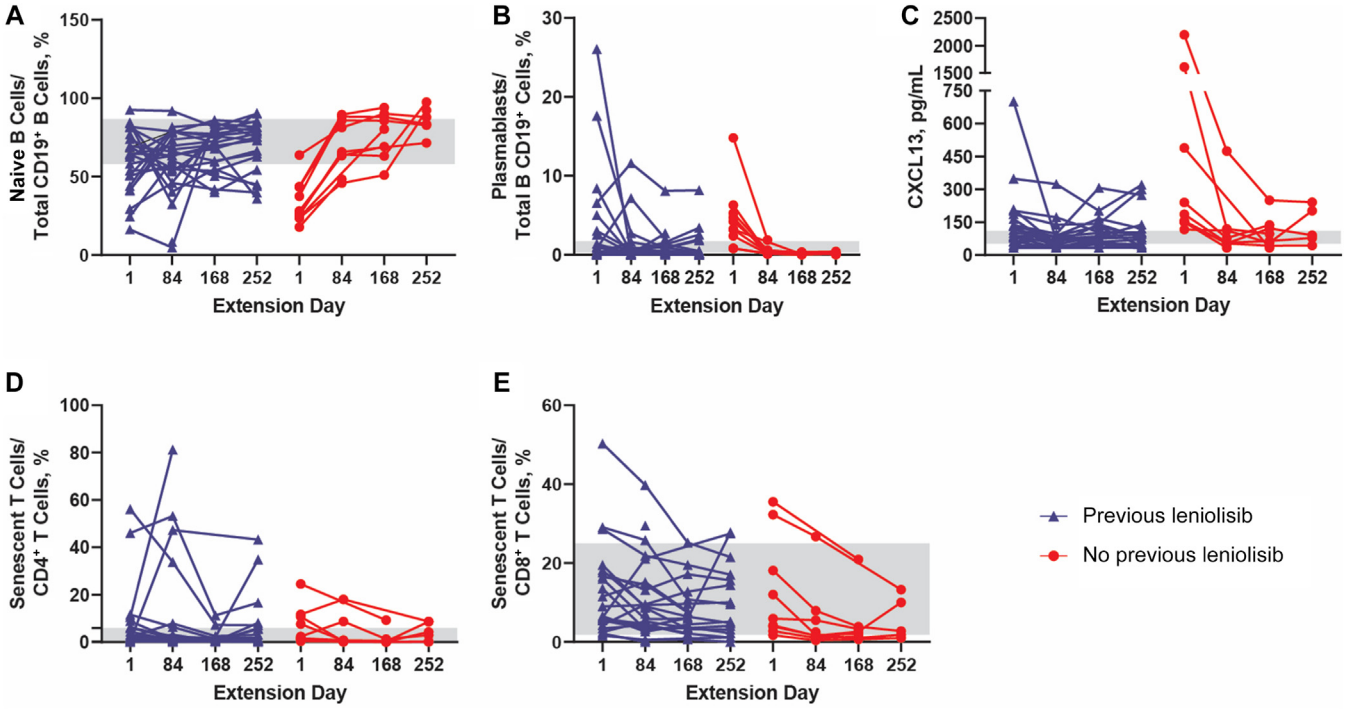

FIG E4. Representative individual results of B- and T-cell subsets compared between patients with and without previous leniolisib exposure. **A**, Individual naive B-cell percentages over time in patients with and without previous leniolisib exposure (n values for ED 1, ED 84, ED 168, and ED 252 for each group: previous leniolisib exposure, 24, 25, 20, 21; no previous leniolisib exposure, 10, 9, 8, 6). **B**, Individual plasmablast B-cell percentages over time in patients with and without previous leniolisib exposure (n values for ED 1, ED 84, ED 168, and ED 252 for each group: previous leniolisib exposure, 24, 24, 20, 17; no previous leniolisib exposure, 10, 9, 8, 6). **C**, Individual CXCL13 values over time in patients with and without previous leniolisib exposure (n values for ED 1, ED 84, ED 168, and ED 252 for each group: previous leniolisib exposure, 21, 20, 18, 20; no previous leniolisib exposure, 9, 8, 8, 5). **D**, Individual $CD4^+$ senescent T-cell percentages over time in patients with and without previous leniolisib exposure (n values for ED 1, ED 84, ED 168, and ED 252 for each group: previous leniolisib exposure, 22, 23, 20, 18; no previous leniolisib exposure, 9, 9, 8, 6). **E**, Individual $CD8^+$ senescent T-cell percentages over time in patients with and without previous leniolisib exposure (n values for ED 1, ED 84, ED 168, and ED 252 for each group: previous leniolisib exposure, 21, 23, 20, 16; no previous leniolisib exposure, 9, 9, 8, 5). Gray-shaded boxes indicate normal range values for respective measurements.

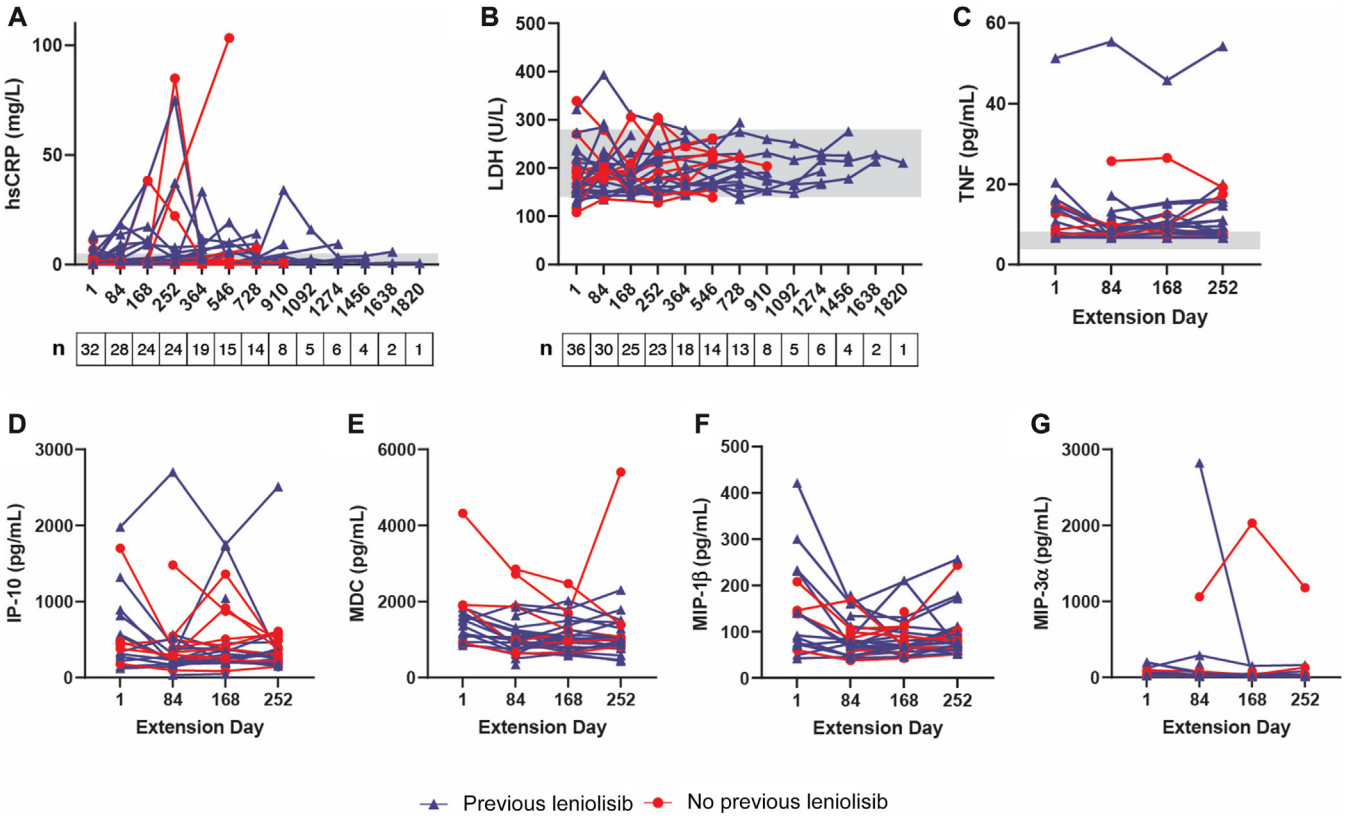

FIG E5. Changes in inflammatory markers over time. **A**, hsCRP levels from individual patients over time (n = 32). **B**, LDH levels from individual patients over time (n = 36). **C**, TNF levels from individual patients over time (n values for ED 1, ED 84, ED 168, and ED 252: 17, 28, 28, 26). **D**, Individual levels of IP-10 over time (n values for ED 1, ED 84, ED 168, and ED 252: 17, 29, 27, 25). **E**, MDC levels from individual patients over time (n values for ED 1, ED 84, ED 168, and ED 252: 17, 29, 27, 26). **F**, Individual patient levels of MIP-1β over time (n values for ED 1, ED 84, ED 168, and ED 252: 17, 29, 27, 26). **G**, Individual patient levels of MIP-3α over time (n values for ED 1, ED 84, ED 168, and ED 252: 15, 24, 23, 21). Gray-shaded boxes indicate normal range values for respective measurements. *hsCRP*, High-sensitivity C-reactive protein; *IP-10*, IFN-γ–induced protein 10; *LDH*, lactose dehydrogenase; *MDC*, macrophage-derived chemokine; *MIP-1β/3α*, macrophage inflammatory protein 1β/3α.

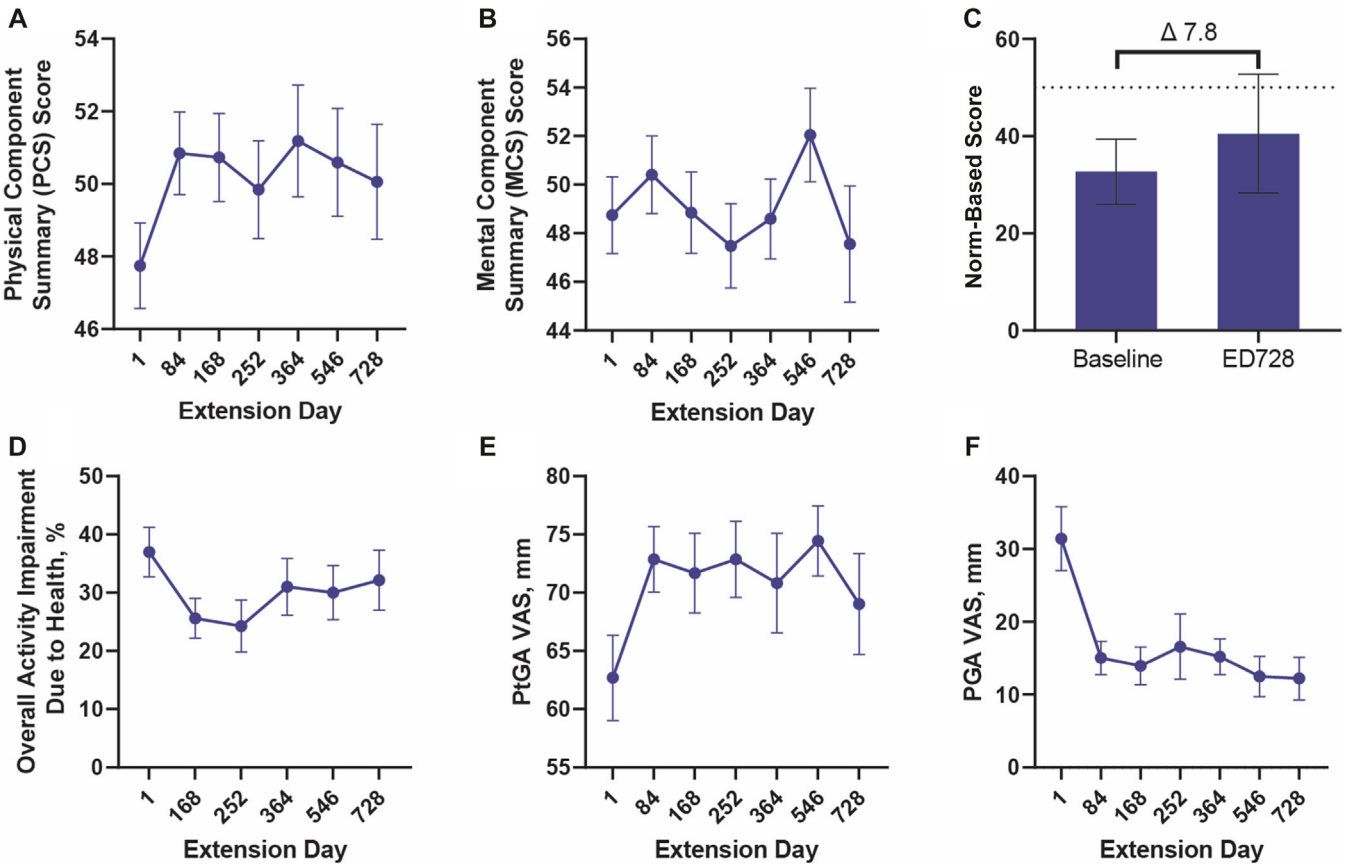

FIG E6. Changes in health-related quality of life over time. **A**, Mean aggregated physical components score over time. **B**, Mean aggregated mental components score over time. **C**, Average norm-based score of general health at baseline and ED 728 (n = 12) (n values for ED 1, ED 84, ED 168, ED 252, ED 364, ED 546, and ED 728 for Fig E6, *A* and *B*: 31, 32, 26, 25, 22, 22, 18). **D**, Mean percentage of the overall activity impairment due to health over time (n values for ED 1, ED 168, ED 252, ED 364, ED 546, and ED 728: 33, 25, 21, 20, 20, 14). **E**, Mean patient general assessment of well-being (PtGA) VAS scores over time (n values for ED 1, ED 84, ED 168, ED 252, ED 364, ED 546, and ED 728: 37, 37, 31, 30, 25, 24, 18). **F**, Mean physician general assessment of disease activity (PGA) VAS scores over time (n values for ED 1, ED 84, ED 168, ED 252, ED 364, ED 546, and ED 728: 31, 32, 25, 26, 25, 25, 17). *VAS*, Visual analog scale.

TABLE E1. SAEs in patients with and without previous leniolisib exposure

	Incidence of AEs, n (%)	
SAEs by preferred term	**Previous leniolisib exposure**	**No previous leniolisib exposure**
Cardiac arrest	1 (3.8)	0
Abdominal pain	0	2 (18.2)
Anal fissure	0	1 (9.1)
Colitis	0	1 (9.1)
Hematochezia	0	1 (9.1)
Vomiting	0	1 (9.1)
Facial pain	0	1 (9.1)
Pyrexia	0	1 (9.1)
Soft tissue abscess	1 (3.8)	0
Acute sinusitis	0	1 (9.1)
Parotitis	0	1 (9.1)
Periorbital cellulitis	0	1 (9.1)
Pneumonia	1 (3.8)	0
Sinusitis	0	1 (9.1)
Increased ALT	0	2 (18.2)
Increased AST	0	1 (9.1)
Dehydration	0	1 (9.1)
Hypocalcemia	1 (3.8)	0
Reactive arthritis	1 (3.8)	0
Aspiration	1 (3.8)	0
Orthostatic hypotension	1 (3.8)	0

TABLE E2. Change in mean percentages of lymphocyte subsets at ED 252 from ED 1

Lymphocyte subsets	Mean percentage of cells over parent cells, % (SEM)		Change in mean percentage as of ED 252 from ED 1 (%)
	ED 1	ED 252	
Naive B cells	52.33 (3.79)	74.62 (3.48)	22.29
Transitional B cells	21.66 (3.21)	9.59 (1.64)	−12.07
Plasmablasts	3.58 (0.97)	0.92 (0.38)	−2.66
Mature B cells	73.59 (4.88)	80.09 (5.37)	6.5
PD-1$^+$ CD4$^+$ T cells	23.61 (4.17)	16.35 (4.30)	−7.26
PD-1$^+$ CD8$^+$ T cells	24.78 (4.04)	23.24 (5.10)	−1.54
CD4$^+$ senescent T cells	7.49 (2.30)	6.54 (2.22)	−0.95
CD8$^+$ senescent T cells	13.00 (2.18)	9.24 (1.89)	−3.76
CD4$^+$ T cells	41.25 (2.43)	45.78 (2.29)	4.53
CD8$^+$ T cells	50.50 (2.27)	34.74 (4.04)	−15.76
CD4$^+$ naive T cells	16.39 (2.38)	17.10 (3.32)	0.71
CD8$^+$ naive T cells	22.72 (2.54)	28.70 (3.31)	5.98
CD4$^+$ central memory T cells	54.46 (2.12)	56.31 (2.96)	1.85
CD8$^+$ central memory T cells	22.65 (2.20)	24.32 (2.97)	1.67
CD4$^+$ effector memory T cells	29.41 (1.68)	25.72 (1.70)	−3.69
CD8$^+$ effector memory T cells	37.51 (2.58)	33.05 (3.16)	−4.46