

A First-in-Human Phase 1/2 Study of Amulirafusp Alfa (IMM0306), a CD20-Targeted CD47-SIRP α Fusion Protein, in Relapsed or Refractory B-Cell Non-Hodgkin Lymphoma

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Abstract

Amulirafusp alfa (IMM0306) consists of a fusion protein that incorporates the CD47-binding domain derived from signal-regulatory protein alpha (SIRP α) fused to a CD20 monoclonal antibody on each of the heavy chains. This investigation examined the safety profile and initial therapeutic activity of amulirafusp alfa in individuals with relapsed or refractory (r/r) B-cell non-Hodgkin lymphoma (B-NHL). Eligible participants included those with CD20-positive r/r B-NHL who had progressed after at least 2 prior treatment regimens. The dosing schedule began with a single administration of amulirafusp alfa during the initial 2-week period, followed by weekly intravenous infusions of the same dose during successive 4-week treatment cycles. Primary objectives focused on characterizing the agent's safety, identifying the maximum tolerated dose (MTD), and establishing the recommended phase 2 dose (RP2D). From May 22, 2020, to February 10, 2022, the trial enrolled 48 patients with r/r B-NHL who received amulirafusp alfa at doses ranging from 40 to 2000 $\mu\text{g/kg}$. By the data cutoff of April 18, 2024, no dose-limiting toxicities had emerged, and the MTD had not been determined. The 2000 $\mu\text{g/kg}$ dose level was selected as the RP2D. TRAEs of any grade affected all 48 patients (100%), while grade 3 or higher events were recorded in 33 patients (68.8%). The predominant grade 3 or higher TRAEs comprised lymphopenia (28/48, 58.3%), leukopenia (10/48, 20.8%), neutropenia (9/48, 18.8%), and anemia (5/48, 10.4%) and in the subgroup receiving 800–2000 $\mu\text{g/kg}$, objective response rates reached 41.2% (7/17, 95% confidence interval [CI] 18.4–67.1) among patients with follicular lymphoma and 33.3% (2/6, 95% CI 3.7–71.0) among those with marginal zone lymphoma. Amulirafusp alfa demonstrated an acceptable safety profile and encouraging early clinical activity in r/r B-NHL, thereby justifying continued development in subsequent studies.

Keywords: Amulirafusp alfa, IMM0306, CD47-SIRP α , CD20-positive B-cell non-Hodgkin lymphoma, Relapsed or refractory lymphoma, Phase 1/2 trial

Introduction

According to the 2019 Global Burden of Disease study, non-Hodgkin lymphoma (NHL) exhibits an incidence of

5.73 per 100 000 individuals and a mortality rate of 3.19 per 100 000 [1]. For the vast majority of B-cell non-Hodgkin lymphoma (B-NHL) subtypes, the established frontline approach involves CD20 monoclonal antibodies (mAbs) combined with standard chemotherapy, which yields prolonged survival for most patients [2]. Despite this, individuals whose disease proves primarily refractory or recurs—typically within the initial two years following therapy—face markedly inferior outcomes [3-5]. Recent advances, such as chimeric antigen receptor T-cell (CAR-T) therapy [6-8] and CD3xCD20 bispecific antibodies [9-11], have improved

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the therapeutic landscape for relapsed or refractory (r/r) B-NHL. Nevertheless, these modalities are frequently associated with significant toxicities stemming from T-cell engagement, most notably cytokine release syndrome [12, 13]. Furthermore, CAR-T treatment requires specialized infrastructure and expertise, limiting its accessibility to select advanced centers [14]. Thus, novel agents offering improved safety and efficacy remain critically needed for this challenging population. CD47 blockade has emerged as an innovative strategy against various malignancies. This transmembrane protein is upregulated on malignant cells and inhibits phagocytosis by interacting with signal-regulatory protein alpha (SIRP α) on myeloid cells, thereby transmitting a “don’t eat me” signal [15]. Both the CD47-directed antibody magrolimab (Hu5F9-G4) and the SIRP α fusion protein TTI-621 have shown acceptable tolerability and single-agent activity in r/r B-NHL [16, 17]. Combining CD47-targeted agents with rituximab can potentiate phagocytic clearance and tumor elimination [18]. Supporting this, the magrolimab–rituximab doublet produced meaningful responses in a phase 1b evaluation involving patients with r/r B-NHL [19].

Amulirafusp alfa (IMM0306), developed by ImmuneOnco Biopharmaceuticals (Shanghai) Inc. in Shanghai, China, is a bispecific fusion protein that joins the CD47-binding portion of SIRP α to a CD20 monoclonal antibody framework on both heavy chains [20]. This construct enables strong simultaneous engagement of CD20-expressing and CD47-expressing cells [20]. Its markedly higher binding affinity for CD20 over CD47 promotes selective targeting of dual-positive lymphoma cells while sparing normal tissues, thereby attenuating CD47-related toxicities such as anemia and thrombocytopenia [15, 20]. In preclinical xenograft models, amulirafusp alfa demonstrated robust antitumor effects by concurrently stimulating macrophage phagocytosis and NK-cell cytotoxicity through SIRP α blockade and Fc γ R-mediated activation [20].

These results provided the rationale for launching a first-in-human phase 1/2 trial evaluating intravenous amulirafusp alfa in patients with r/r B-NHL. This manuscript presents data from the dose-escalation stage of the study, with primary goals focused on safety assessment and defining the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D) for amulirafusp alfa.

Materials and Methods

Study design and patients

The present multicenter, open-label, first-in-human phase 1/2 clinical trial was undertaken to assess the safety, tolerability, pharmacokinetic characteristics, immunogenicity, and initial therapeutic effects of amulirafusp alfa. Dose escalation in the phase 1 segment followed two successive strategies. An accelerated titration scheme was first applied to the 40, 100, and 250 μ g/kg dose cohorts. This was then succeeded by a standard “3 + 3” design for the remaining higher dose levels.

Inclusion required patients to be adults of 18 years or above with histologically verified relapsed or refractory B-NHL after prior therapy. They also needed an Eastern Cooperative Oncology Group (ECOG) performance status (PS) score of 0-2, sufficient organ function, at least one measurable or assessable lesion, and an anticipated survival of 3 months or longer. Exclusion applied to those with active central nervous system involvement by lymphoma, a history of allogeneic hematopoietic or solid organ transplantation, autologous stem cell transplantation within the preceding 100 days, ongoing or recurrent autoimmune disorders, prior receipt of CD47-targeting antibodies or SIRP α -based agents, chronic treatment with antiplatelet medications including clopidogrel, aspirin, or comparable nonsteroidal anti-inflammatory drugs, or the need for systemic corticosteroids above 10 mg daily prednisone equivalent or other immunosuppressants within one week before starting treatment or at any point during the trial.

All procedures complied with recognized Good Clinical Practice principles and the Declaration of Helsinki. Institutional review boards and ethics committees at each site approved the protocol and any modifications. Written informed consent was secured from every enrolled individual. The trial was registered on ClinicalTrials.gov under the number NCT05805943.

Procedures

In the phase 1 dose-escalation stage, participants first underwent a single-dose observation phase spanning the initial two weeks. This was followed by repeated weekly intravenous dosing of the assigned amount within 4-week cycles. Treatment continued until patients experienced prohibitive toxicity, disease progression, death, withdrawal of consent, investigator-initiated discontinuation, initiation of other anticancer

interventions, reaching the 50-week maximum treatment period, or overall study closure. Dose-limiting toxicity (DLT) evaluation occurred over the first four weeks. Qualifying DLTs consisted of particular hematologic issues such as grade 3 febrile neutropenia, prolonged grade 4 neutropenia beyond five days, grade 3 thrombocytopenia involving bleeding or requiring transfusion support, grade 4 thrombocytopenia, hemolysis at grade 3 or above, or any other grade 4 blood-related toxicity. Non-hematologic DLTs included any grade 4 event, grade 3 toxicities affecting major organs (cardiac, pulmonary, gastrointestinal, hepatic, renal, or neurologic), grade 3 cytokine release syndrome that failed to improve to grade 1 or baseline levels within 7 days, and grade 3 fatigue lasting for more than 14 days. Core primary endpoints included safety monitoring and the establishment of the maximum tolerated dose (MTD) and the recommended phase 2 dose (RP2D) for amulirafusp alfa. Secondary objectives included pharmacokinetic evaluation, immunogenicity assessment, and efficacy outcomes. Exploratory goals addressed pharmacodynamic markers, immune-related indicators, and levels of CD47 receptor occupancy, among others.

All adverse events were coded according to MedDRA version 27.0, and severity was assigned using CTCAE version 5.0. The MTD was the highest dose at which DLT incidence remained at or below 1 patient per group of 6. Determination of the RP2D relied on combined consideration of safety observations, drug exposure levels, pharmacodynamic responses, and signs of clinical benefit.

Pharmacokinetic profiling relied on serial blood collections performed after dosing during the single-dose phase at cycle 0 day 1 (C0D1), cycle 0 day 2 (C0D2), cycle 0 day 3 (C0D3), cycle 0 day 4 (C0D4), cycle 0 day 5 (C0D5), and cycle 0 day 8 (C0D8). In the multiple-dose phase, sampling occurred on cycle 1 day 1 (C1D1), cycle 1 day 8 (C1D8), cycle 1 day 15 (C1D15), cycle 1 day 22 (C1D22), and equivalent points in subsequent cycles. Key single-dose parameters include the maximum observed concentration (C_{max}), the time to maximum concentration (T_{max}), and the area under the concentration-time curve from baseline to the final measurable point (AUC_{0-last}). When data were adequate, additional metrics, including the area under the curve extrapolated to infinity (AUC_{0-inf}), elimination half-life ($t_{1/2}$), volume of distribution (V), and systemic clearance (CL), were derived. For multiple dosing,

parameters included steady-state trough concentration ($C_{min,ss}$), steady-state peak concentration ($C_{max,ss}$), steady-state average concentration ($C_{av,ss}$), area under the curve over the dosing interval (AUC_{0-tau}), T_{max} , accumulation ratios derived from C_{max} (Rac_C_{max}) and AUC_{0-tau} (Rac_AUC_{0-tau}), along with steady-state volume of distribution (V_{SS}), steady-state clearance (CL_{ss}), and half-life when calculable.

Assessment of immunogenicity focused on the occurrence of anti-drug antibodies (ADAs). Samples testing positive for ADAs were subsequently analyzed for titer levels and the presence of neutralizing antibodies (NAbs).

Response evaluation in chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma (SLL) was performed according to the 2008 International Workshop on Chronic Lymphocytic Leukemia (IWCLL) guidelines [21]. In contrast, other B-NHL subtypes were evaluated according to the Lugano criteria [22]. The best objective response (BOR) was recorded as the most favorable outcome observed from treatment commencement until confirmed disease progression (PD), death, or the start of new therapy. Objective response rate (ORR) was defined as the proportion of patients who achieved a complete response (CR) or partial response (PR) as their BOR. Disease control rate (DCR) included those with CR, PR, or stable disease (SD). Upon initial evidence of progression, continuation of therapy was permitted pending confirmation with lymphoma response to immunomodulatory therapy criteria (LYRIC) [23] to distinguish pseudoprogression. Progression-free survival (PFS) was measured as the interval from the initial dose to the first confirmed PD or death from any cause. Duration of response (DoR) spanned from initial CR or PR documentation to subsequent PD. Overall survival (OS) was defined as the time from first dosing to death, regardless of cause.

Additional exploratory work examined pharmacodynamic responses and potential predictive biomarkers of efficacy and safety by analyzing peripheral blood specimens collected at screening and on-treatment time points. Flow cytometry determined CD47 receptor occupancy on circulating lymphocytes and tracked absolute CD19-positive B-cell counts to gauge B-cell depletion. Serum cytokine profiling was conducted using enzyme-linked immunosorbent assay.

Statistical analysis

Analyses of safety, pharmacokinetics, and efficacy were conducted exclusively using descriptive statistics, without performing any formal statistical hypothesis tests. Summary statistics for categorical variables were expressed as patient counts and corresponding percentages within each category. For continuous variables, results were presented as the number of non-missing observations, medians, and ranges (minimum to maximum). Calculation of 95% confidence intervals (CIs) for the objective response rate (ORR) utilized the Clopper-Pearson exact method. Kaplan–Meier estimates were applied to time-to-event endpoints, with median values and associated 95% CIs reported. Subgroup assessments of response rates and survival outcomes, stratified by baseline characteristics, followed the same descriptive approach as for the entire study population. The full analysis set (FAS) included all patients who received at least 1 dose of amulirafusp alfa and served as the population for baseline characteristic summaries. Safety evaluations were restricted to the safety set (SS), which comprised all FAS patients with available post-dose safety data. The pharmacokinetic concentration set (PKCS) consisted of FAS patients who had at least one measurable serum concentration record. The pharmacokinetic parameter set (PKPS) included FAS patients who provided at least one evaluable pharmacokinetic parameter, after excluding those with substantial protocol violations that could compromise parameter validity. The anti-drug antibody set (ADAS) encompassed SS patients with at least one valid immunogenicity assessment, excluding individuals who

tested positive for anti-drug antibodies at baseline. The dose-limiting toxicity set (DLTS) included all FAS patients who either completed the designated DLT observation period or developed a DLT during that interval. Tumor response evaluations were performed in the efficacy set (EFS), defined as FAS patients with measurable or evaluable baseline lesions who underwent at least one post-baseline disease assessment.

All statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., North Carolina, US). Non-compartmental analysis (NCA) for deriving pharmacokinetic parameters was performed using Phoenix software version 8.2 (Certara L.P., New Jersey, US).

Results and Discussion

Patient baseline characteristics

Between May 22, 2020, and February 10, 2022, 48 patients diagnosed with B-NHL were recruited from seven institutions in China and treated with amulirafusp alfa at one of eight progressively increasing dose levels (**Figure 1**). The initial nine participants were enrolled under an accelerated titration scheme and received 40 µg/kg (n = 1), 100 µg/kg (n = 2), or 250 µg/kg (n = 6). Later expansion cohorts were formed at 1200 µg/kg (n = 10), 1600 µg/kg (n = 9), and 2000 µg/kg (n = 13) to collect additional safety, pharmacokinetic, pharmacodynamic, and preliminary antitumor activity data.

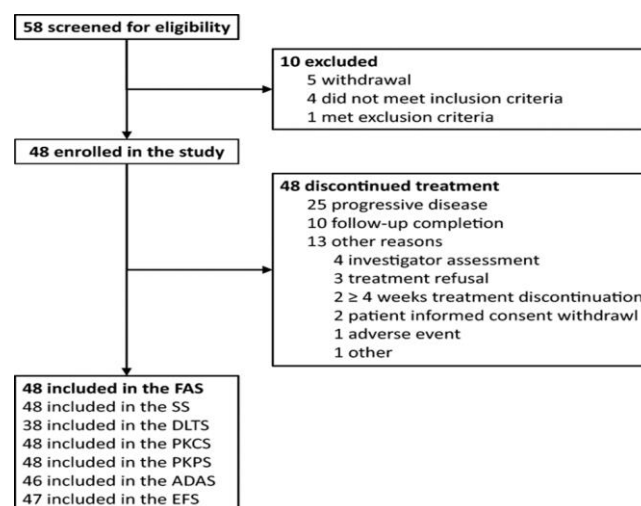


Figure 1. Flowchart of the study. ADAS, anti-drug antibody set; DLTS, dose-limiting toxicity set; EFS, efficacy set; FAS, full analysis set; PKCS, pharmacokinetic concentration set; PKPS, pharmacokinetic parameter set; SS, safety set

Demographic and disease-related baseline features for the study population are presented in **Table 1**. Within the cohort of 48 patients, follicular lymphoma (FL) accounted for 20 cases (41.7%), diffuse large B-cell lymphoma (DLBCL) for 15 cases (31.3%), marginal zone lymphoma (MZL) for 7 cases (14.6%), and mantle cell lymphoma (MCL) for 3 cases (6.3%). The remaining three patients (6.3%) included one with blastoid variant MCL, one with histologic transformation from indolent B-cell lymphoma to DLBCL, and one with CLL/SLL, classified per the 2016 World Health Organization update on lymphoid neoplasms [24]. Males constituted 30 patients (62.5%), and the median age across the group was 56 years (range 27–76). Nearly all participants (46/48, 93.9%) presented with an ECOG performance

status of 0 or 1. Prior exposure to anti-CD20 monoclonal antibodies was universal. Patients had undergone a median of 3 prior lines of therapy (range 1–8), with the majority (25/48, 52.1%) exhibiting refractoriness to their most recent systemic treatment. One patient (2.1%) with transformed lymphoma had a history of autologous hematopoietic stem cell transplantation. Two FL patients had previously received CD3xCD19 bispecific antibody therapy, and one DLBCL patient had received CAR-T therapy. Among FL patients (20/48, 41.7%), the median number of previous systemic therapies was 2 (range 1–7). DLBCL patients (15/48, 31.3%) had received a median of 4 prior lines (range 2–6), while MZL patients (7/48, 14.6%) had a median of 2 prior lines (range 1–2).

Table 1. Demographic and clinical characteristics at baseline of patients in the FAS (n = 48).

Characteristic	Overall population ^a (n = 48)	MZL ^b (n = 7)	DLBCL ^a (n = 15)	FL (n = 20)
Age, median years (range)	56 (27–76)	56 (41–64)	57 (27–69)	59 (35–76)
Sex, n (%)				
Female	18 (37.5)	2 (28.6)	6 (40.0)	9 (45.0)
Male	30 (62.5)	5 (71.4)	9 (60.0)	11 (55.0)
ECOG performance status, n (%)				
0	15 (31.3)	5 (71.4)	3 (20.0)	5 (25.0)
1	31 (64.6)	2 (28.6)	11 (73.3)	14 (70.0)
2	2 (4.2)	0	1 (6.7)	1 (5.0)
Lugano stage at study entry^d, n (%)				
I	0	0	0	0
II	12 (25.5)	2 (28.6)	6 (40.0)	3 (15.0)
II with bulky lesions	0	0	0	0
III	13 (27.7)	2 (28.6)	3 (20.0)	8 (40.0)
IV	22 (46.8)	3 (42.9)	6 (40.0)	9 (45.0)
Duration since diagnosis, median months (range)	38.2 (6.7–103.8)	78.00 (10.8–99.9)	18.8 (7.9–86.2)	39.3 (6.7–97.1)
Number of prior treatment lines, median (range)	2.5 (1–8)	2.0 (1–2)	4.0 (2–6)	2.0 (1–7)
Prior treatment lines, n (%)				
≤2 previous lines	24 (50.0)	7 (100)	3 (20.0)	13 (65.0)
≥3 previous lines	24 (50.0)	0	12 (80.0)	7 (35.0)
CD20 expression positive, n (%)	47 (97.9) ^e	7 (100%)	14 (93.3)	20 (100)
Disease status at enrollment, n (%)				
Relapsed	23 (47.9)	3 (42.9)	5 (33.3)	12 (60.0)
Refractory	25 (52.1)	4 (57.1)	10 (66.7)	8 (40.0)

- ^a: Including 1 patient with primary bone DLBCL; ^b: Including 1 patient with MALT-L; ^c: Including 3 (6.3%) patients with MCL and 3 (6.3%) patients with blastoid variant of MCL, histologic transformation of indolent B-cell lymphoma to DLBCL, and CLL/SLL, respectively, except for patients with FL, DLBCL, and MZL; ^d: One patient with CLL/SLL was not suitable for Lugano staging system; ^e: One patient was diagnosed with CD20-positive at the initial pathological diagnosis while CD20-negative at the last pathology diagnosis before enrollment
- CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma, DLBCL: diffuse large B-cell lymphoma, FAS: full analysis set, FL: follicular lymphoma, MALT-L: mucosa-associated lymphoid tissue lymphoma, MCL: mantle cell lymphoma, MZL: marginal zone lymphomas, n: number of patients included in statistical analyses, no.: number of patients

Safety

By the data cutoff on April 18, 2024, the entire study population (48/48, 100%) had received at least 1 dose of amulirafusp alfa and was therefore evaluable in the safety set (SS). Thirty-eight patients (79.2%) met criteria for inclusion in the dose-limiting toxicity set (DLTS). In the multiple-dose phase, the median number of doses administered was 7.5 (range 1–87), with a median duration of exposure of 1.6 months (range 0.03–21.8) and a median relative dose intensity of 98.9% (range 50.0–107.5). The agent was generally well tolerated across the tested dose range, with no DLTs observed. As a result, the maximum tolerated dose (MTD) was not established. All patients (48/48, 100%) discontinued study treatment for the following reasons: disease progression (25/48, 52.1%), completion of protocol-defined treatment (10/48, 20.8%), investigator discretion (4/48, 8.3%), patient decision to stop (3/48, 6.3%), treatment hold longer than 4 weeks (2/48, 4.2%), withdrawal of consent (2/48, 4.2%), adverse event (1/48, 2.1%), and miscellaneous reasons (1/48, 2.1%).

Treatment-emergent adverse events (TEAEs) as well as treatment-related adverse events (TRAEs) were documented in every participant (48/48, 100%) (**Table**

2). Commonly observed TRAEs included decreased white blood cell (WBC) count (32/48, 66.7%), anemia (31/48, 64.6%), decreased lymphocyte count (29/48, 60.4%), decreased absolute neutrophil count (ANC) (23/48, 47.9%), decreased platelet count (23/48, 47.9%), and infusion-related reactions (17/48, 35.4%). Grade 3 or higher TEAEs affected 38 patients (79.2%), while grade 3 or higher TRAEs occurred in 33 patients (68.8%). The leading $\geq$ grade 3 TRAEs comprised decreased lymphocyte count (28/48, 58.3%), decreased WBC count (10/48, 20.8%), decreased ANC (9/48, 18.8%), anemia (5/48, 10.4%), infectious pneumonia (3/48, 6.3%), elevated gamma-glutamyltransferase (2/48, 4.2%), decreased platelet count (2/48, 4.2%), and sinus tachycardia (1/48, 2.1%). Treatment was permanently stopped in one patient (2.1%) due to grade 4 thrombocytopenia considered related to amulirafusp alfa. Serious adverse events were reported in 14 patients (29.2%), with 6 cases (12.5%) deemed drug-related; these included pneumonia (4/48, 8.3%), decreased platelet count (1/48, 2.1%), and chest pain (1/48, 2.1%). The majority of TEAEs resolved or improved following appropriate supportive measures, dose interruptions, or both, leaving no lasting effects.

Table 2. TRAEs that occurred in at least 10% of patients in the SS (n = 48).

Adverse event	Grade 4, n (%)	Grade 3, n (%)	Grade 1–2, n (%)	All grades, n (%)
Any adverse event	23 (47.9%)	10 (20.8%)	15 (31.3%)	48 (100%)
Decreased white blood cell count	0	10 (20.8%)	22 (45.8%)	32 (66.7%)
Anemia	0	5 (10.4%)	26 (54.2%)	31 (64.6%)
Reduced lymphocyte count	22 (45.8%)	6 (12.5%)	1 (2.1%)	29 (60.4%)
Decreased platelet count	1 (2.1%)	1 (2.1%)	21 (43.8%)	23 (47.9%)
Decreased absolute neutrophil count	1 (2.1%)	8 (16.7%)	14 (29.2%)	23 (47.9%)
Infusion-related reactions	0	0	17 (35.4%)	17 (35.4%)
Increased lactate dehydrogenase	0	0	9 (18.8%)	9 (18.8%)
Increased alanine aminotransferase	0	0	8 (16.7%)	8 (16.7%)
Pyrexia	0	0	8 (16.7%)	8 (16.7%)
Increased gamma-glutamyl transferase	0	2 (4.2%)	6 (12.5%)	8 (16.7%)
Increased α -hydroxybutyrate dehydrogenase	0	0	7 (14.6%)	7 (14.6%)
Increased aspartate aminotransferase	0	0	7 (14.6%)	7 (14.6%)
Hypercholesterolemia	0	0	6 (12.5%)	6 (12.5%)
Increased C-reactive protein	0	0	5 (10.4%)	5 (10.4%)

1. AE: adverse event, ALT: alanine aminotransferase, ANC: absolute neutrophil count, AST: aspartate aminotransferase, α -HBDH: alpha hydroxybutyrate dehydrogenase, γ -GT: Gamma-glutamyltransferase, LDH: Lactate dehydrogenase, n: number of patients included in statistical analyses, no.: number of patients, SS: safety set, TRAEs: Treatment-related adverse events, WBC: White blood cell

No deaths were recorded during active treatment. Eleven patients (22.9%) died in the post-discontinuation follow-up period. The principal cause was disease progression (7/11, 63.6%). Other causes included cardiac arrest (1/11,

9.1%) and severe pneumonia (1/11, 9.1%). The cause of death could not be determined for two patients (18.2%) who died while under survival monitoring.

Pharmacokinetic

The pharmacokinetic concentration set (PKCS) and pharmacokinetic parameter set (PKPS) both comprised all 48 enrolled patients. Following a single dose, amulirafusp alfa exposure rose proportionally with dose escalation from 100 to 2000 µg/kg. Mean terminal half-life ($t_{1/2}$) values ranged between 18.8 and 46.3 hours, mean volume of distribution at steady state (VSS) between 9.65 and 34.5 L, and mean clearance (CL) between 0.255 and 1.08 L/h, with clearance tending to decline as dose increased.

With repeated weekly administration, drug exposure similarly increased across the 100–2000 µg/kg dose levels. Mean $t_{1/2}$ ranged from 15.8 to 42.2 hours, mean steady-state clearance (CL_{ss}) from 0.234 to 1.17 L/h, mean VSS from 8.51 to 24.6 L, mean accumulation ratio based on maximum concentration (Rac_C_{max}) from 0.665 to 1.47, and mean accumulation ratio based on area under the curve (Rac_AUC_{0-tau}) from 1.03 to 18.7. These parameters demonstrated limited drug accumulation when dosed once weekly. Trough concentrations generally stabilized after four weekly doses in each cohort. Overall, exposure-response assessments confirmed nonlinear pharmacokinetic properties of amulirafusp alfa within the evaluated dose range.

Immunogenicity

Forty-six patients (95.8%) formed the anti-drug antibody set (ADAS). Anti-drug antibodies (ADA) were detected in two individuals (one receiving 250 µg/kg and one receiving 2000 µg/kg). ADA positivity did not meaningfully affect safety, pharmacokinetics, or treatment efficacy.

Efficacy

Forty-seven patients (97.9%) were included in the efficacy set (EFS), comprising 46 patients assessed under Lugano criteria and 1 under CLL/SLL criteria. The single patient in the CLL/SLL EFS (from the 1200 µg/kg group) exhibited progressive disease (PD) as the best overall response.

Among the 46 patients in the Lugano EFS, five achieved complete response (CR), five achieved partial response (PR), and 14 had stable disease (SD) (**Table 3**). This yielded an objective response rate (ORR) of 21.7% (10/46, 95% CI: 10.9–36.4) and a disease control rate (DCR) of 52.2% (24/46, 95% CI: 36.9–67.1). The five CRs included one MZL patient at 800 µg/kg, three FL patients at 1200 µg/kg, and one FL patient at 2000 µg/kg. The five PRs included one mucosa-associated lymphoid tissue lymphoma patient at 1200 µg/kg, one FL patient at 1600 µg/kg, and two FL patients and one primary bone DLBCL patient at 2000 µg/kg. Eleven patients demonstrated a reduction of at least 50% in the sum of the product of lesion diameters (**Figures 2a and 2b**).

Table 3. Objective responses to amulirafusp alfa in the Lugano EFS (n = 46).

Response	Total (n = 46), n (%)	MZL (n = 7), n (%)	DLBCL (n = 14), n (%)	FL (n = 20), n (%)
Objective response	10 (21.7)	2 (28.6)	1 (7.1)	7 (35.0)
Disease control	24 (52.2)	5 (71.4)	5 (35.7)	13 (65.0)
CR	5 (10.9)	1 (14.3)	0	4 (20.0)
PR	5 (10.9)	1 (14.3) ^b	1 (7.1) ^a	3 (15.0)
SD	14 (30.4)	3 (42.9)	4 (28.6)	6 (30.0)
PD	22 (47.8)	2 (28.6)	9 (64.3)	7 (35.0)

1. ^a: This is a patient with primary bone DLBCL; ^b: This is a patient with MALT-L

2. CR: complete response, DLBCL: diffuse large B-cell lymphoma, EFS: efficacy set, FL: follicular lymphoma, MALT-L: mucosa-associated lymphoid tissue lymphoma, MZL: marginal zone lymphomas, n: number of patients included in statistical analyses, PD: progressive disease, PR: partial response, SD: stable disease

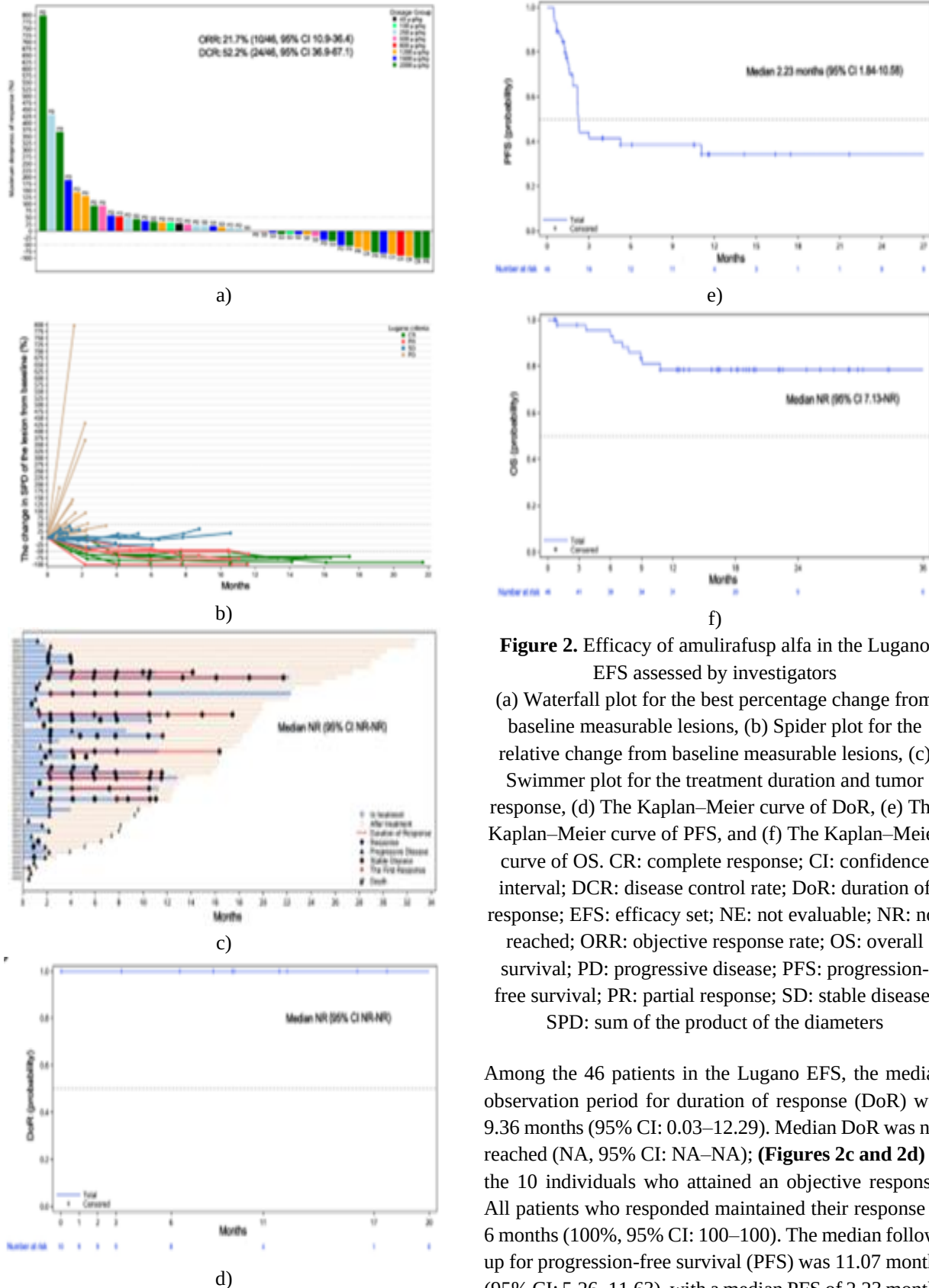


Figure 2. Efficacy of amulirafusp alfa in the Lugano EFS assessed by investigators

(a) Waterfall plot for the best percentage change from baseline measurable lesions, (b) Spider plot for the relative change from baseline measurable lesions, (c) Swimmer plot for the treatment duration and tumor response, (d) The Kaplan–Meier curve of DoR, (e) The Kaplan–Meier curve of PFS, and (f) The Kaplan–Meier curve of OS. CR: complete response; CI: confidence interval; DCR: disease control rate; DoR: duration of response; EFS: efficacy set; NE: not evaluable; NR: not reached; ORR: objective response rate; OS: overall survival; PD: progressive disease; PFS: progression-free survival; PR: partial response; SD: stable disease; SPD: sum of the product of the diameters

Among the 46 patients in the Lugano EFS, the median observation period for duration of response (DoR) was 9.36 months (95% CI: 0.03–12.29). Median DoR was not reached (NA, 95% CI: NA–NA); (**Figures 2c and 2d**) in the 10 individuals who attained an objective response. All patients who responded maintained their response at 6 months (100%, 95% CI: 100–100). The median follow-up for progression-free survival (PFS) was 11.07 months (95% CI: 5.26–11.63), with a median PFS of 2.23 months

(95% CI: 1.84–10.58); **(Figure 2e)**. Estimated PFS rates were 38.5% (95% CI: 23.5–53.5) at 6 months, 34.2% (95% CI: 19.3–49.7) at 12 months, and 34.2% (95% CI: 19.3–49.7) at 18 months. For overall survival (OS), median follow-up extended to 18.30 months (95% CI: 16.20–19.94); **(Figure 2f)**. Median OS was not reached (95% CI: 7.13–NR). OS rates were estimated at 93.0% (95% CI: 79.7–97.7) at 6 months, 78.5% (95% CI: 62.8–88.2) at 12 and 18 months, and 75.0% (95% CI: 12.8–96.1) at 24 months.

Amulirafusp alfa produced signs of meaningful early antitumor activity in CD20-positive relapsed or refractory B-NHL at doses between 800 and 2000 µg/kg, with more notable effects observed in follicular lymphoma (FL) and marginal zone lymphoma (MZL) **(Table 3)**. The ORR in FL patients treated at these dose levels was 41.2% (7/17, 95% CI: 18.4–67.1), while that in MZL patients was 33.3% (2/6, 95% CI: 4.3–77.7). Median PFS for the 17 FL patients in this dose range was 10.58 months (95% CI: 2.20–NA).

Recommended phase 2 dose

Based on an overall appraisal of safety findings, pharmacokinetic data, pharmacodynamic markers, and efficacy signals from the dose-escalation portion, the recommended phase 2 dose (RP2D) was established as 2000 µg/kg.

Pharmacodynamic and exploratory analysis

Treatment with amulirafusp alfa induced substantial B-cell depletion in circulating blood. A swift drop in CD19-positive B-cell numbers occurred immediately after the first infusion at all dose levels examined, reaching a nadir on cycle 1 day 1 (C1D1). Recovery of B-cell counts began on cycle 1 day 22 (C1D22) at 500 µg/kg. It returned to pretreatment values by the end of treatment (EOT). At 800 and 1200 µg/kg, B-cell counts displayed only modest rebound from C1D22 onward while staying generally low during ongoing therapy. In contrast, the 1600 and 2000 µg/kg cohorts maintained extremely low B-cell levels, approaching zero, from C1D22 to EOT, demonstrating the strongest B-cell-depleting activity.

Across the full analysis set of 48 patients, cytokine elevations did not exhibit a clear relationship with administered dose. Changes in interleukin-6, interferon-γ, tumor necrosis factor-α, and interferon-γ-induced protein 10 followed parallel temporal patterns. Cytokine concentrations increased markedly shortly after dosing, attained peak values around 4 hours post-infusion, and

subsequently declined, returning to baseline within approximately 24 hours. No substantial cytokine elevations were detected during repeated dosing cycles. When patients were grouped by best objective response into responders (CR or PR) and non-responders (SD or PD), cytokine profiles showed no meaningful association with clinical benefit. These short-lived cytokine fluctuations did not lead to clinical cytokine release syndrome, consistent with the trial's complete absence of this toxicity.

CD47 receptor occupancy on peripheral lymphocytes peaked rapidly after the initial dose. It declined markedly by 24 hours. During weekly dosing, peak occupancy levels remained similar to those seen after the first administration. The limited and transient engagement of CD47 on normal circulating lymphocytes likely contributes to the favorable safety and tolerability characteristics of amulirafusp alfa.

Although important progress has been made in managing relapsed or refractory B-NHL, significant gaps remain in the availability of therapeutic options that combine safety, effectiveness, and ease of administration. Agents targeting the CD47-SIRPα interaction represent a readily accessible treatment option that may particularly benefit individuals with r/r B-NHL who are unsuitable for, or lack access to, CAR-T cell therapy, bispecific antibodies, or other advanced interventions.

The CD47-SIRPα interaction functions as a key “don't eat me” signal within the innate immune system, suppressing macrophage-mediated phagocytosis of tumor cells and thereby serving as an important immune checkpoint in oncology [25]. Disruption of this pathway not only promotes macrophage engulfment of cancer cells but also facilitates tumor elimination through natural killer cell-mediated antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity, and may even trigger direct apoptotic pathways in malignant cells [26]. Furthermore, CD47-targeted antibodies can enhance dendritic cells antigen presentation, thereby stimulating adaptive antitumor immunity [27]. Magrolimab, the first humanized CD47-directed antibody, demonstrated acceptable tolerability in patients with diverse malignancies during early-phase testing [16]. When combined with rituximab, it produced enhanced clinical responses in DLBCL and FL [19]. In acute myeloid leukemia, including cases harboring TP53 mutations, magrolimab in combination with azacitidine demonstrated promising safety and efficacy in a phase 1b study [28].

Given the broad expression of CD47 across hematopoietic tissues, particularly on erythrocytes and platelets, strategies that simultaneously engage CD47 and tumor-associated antigens can improve selective tumor cell clearance while limiting off-target effects [25]. Accordingly, various bispecific antibodies and fusion proteins have been engineered to target both CD47-SIRPα and other tumor-specific markers, enabling multifaceted antibody-dependent mechanisms of tumor destruction.

To the best of current knowledge, amulirafusp alfa represents the first clinical-stage fusion protein that links the CD47-binding domain of SIRPα to a CD20 monoclonal antibody on both heavy chains. The present study demonstrated that amulirafusp alfa was safe and generally well tolerated when administered weekly at doses reaching 2000 µg/kg in patients with B-NHL. The agent displayed a manageable toxicity profile, with no treatment-related fatalities, no instances of cytokine release syndrome, no dose-limiting toxicities, and no attainment of the maximum tolerated dose. Treatment adherence was high: all 48 patients (100%) completed the single-dose phase without requiring dose modifications. In the multiple-dose phase, 42 patients (87.5%) completed treatment, with dose adjustment needed in only one case (2.4%) due to body weight variation. Observed TRAEs were effectively handled through supportive care, temporary treatment pauses, or both. The predominant grade 3 or higher TRAEs consisted of anticipated on-target hematologic effects, including decreased lymphocyte count (28/48, 58.3%), decreased white blood cell count (10/48, 20.8%), decreased absolute neutrophil count (9/48, 18.8%), and anemia (5/48, 10.4%). Lymphocyte reduction appears linked to the intended antitumor mechanism of amulirafusp alfa. Among patients with grade 3 or higher cytopenias, symptomatic intervention was required in 50% with grade 3 leukopenia, 66.7% with grade 3 or higher neutropenia, 40% with grade 3 anemia, and 100% with grade 3 or higher thrombocytopenia. Most patients receiving supportive measures recovered or experienced symptom resolution without residual effects, typically within 3 days, whereas spontaneous recovery in untreated cases generally occurred within 1 month. Notably, unlike the frequent occurrence of cytokine release syndrome associated with CD3xCD20 bispecific antibodies [9, 10, 29-32], the brief cytokine elevation observed within 24 hours after the first dose of amulirafusp alfa did not result in any clinical cytokine release syndrome in this trial.

Amulirafusp alfa displayed advantageous pharmacokinetic characteristics, including a half-life of approximately 1.5 days. This profile supported platelet count recovery during the weekly dosing interval while sustaining near-complete B-cell depletion at doses exceeding 800 µg/kg. In the current trial, only 1 patient (2.1%) experienced grade 4 thrombocytopenia-related TRAE, necessitating permanent discontinuation of treatment. Among patients receiving 800–2000 µg/kg, 33 of 48 (68.8%) exhibited a prompt but transient reduction in platelet counts at 4 hours post-infusion, with gradual recovery by the third day and return to baseline or grade 1 levels within roughly 1 week after the initial dose.

Although the study was not designed with sufficient statistical power to draw definitive efficacy conclusions, amulirafusp alfa demonstrated single-agent antitumor activity in heavily pretreated individuals with r/r B-NHL, including subtypes such as FL, MZL, MCL, and DLBCL. Among the 10 patients who achieved an objective response, the 26-week DoR rate was 100% (95% CI 100–100). Patients with FL derived notable benefit, with 13 of 20 individuals achieving stable disease or better, including 4 complete responses and 3 partial responses. In the subset of 17 FL patients treated at 800–2000 µg/kg, median PFS reached 10.58 months (95% CI: 2.20–NA). Additionally, one complete response and one partial response were recorded in the two patients with MZL. Disease stabilization was further noted across histologic subtypes, occurring in 6 patients with FL (30.0%), 4 with DLBCL (30.8%), 3 with MZL (50.0%), and 2 with MCL (66.7%). Collectively, these early findings suggest the potential utility of amulirafusp alfa across a range of B-NHL histologic subtypes.

Conclusion

In summary, this first-in-human phase 1 investigation of amulirafusp alfa successfully fulfilled its primary objectives by identifying the RP2D in patients with r/r B-NHL, a group with limited effective treatment alternatives. The observed safety profile, supported by pharmacokinetic and pharmacodynamic data and efficacy signals, supports selecting the 2000 µg/kg once-weekly dose as the RP2D for amulirafusp alfa monotherapy across various B-NHL subtypes. The most appropriate dosing schedule is being refined in the ongoing phase 2 expansion cohort of this trial, which will inform the design of future efficacy studies. It is noteworthy that the dose-escalation portion of this phase

1 trial included only two FL patients who had progressed after CD3xCD19 bispecific antibody therapy and one DLBCL patient who had failed prior CAR-T treatment. Additional insights regarding optimal treatment sequencing and combination strategies with amulirafusp alfa are anticipated from subsequent clinical investigations.

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