Evorpacept plus rituximab for the treatment of relapsed or refractory non-Hodgkin lymphoma: results from the phase I ASPEN-01 study

Tae Min Kim,[1] Nehal J. Lakhani,[2] Jacob Soumerai,[3] Manali Kamdar,[4] Justin F. Gainor,[3] Wells Messersmith,[4] Philip Fanning,[5] Shanhong Guan,[5] Feng Jin,[5] Alison Forgie,[5] Hong I. Wan,[5] Jaume Pons,[5] Sophia S. Randolph[5] and Won Seog Kim[6]

[1]Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul, South Korea; [2]START Midwest, Grand Rapids, MI, USA; [3]Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA, USA; [4]University of Colorado Cancer Center, Aurora, CO, USA; [5]ALX Oncology Inc., South San Francisco, CA, USA and [6]Sungkyunkwan University School of Medicine, Samsung Medical Center, Seoul, Korea

Correspondence: S.S. Randolph
sophia@alxoncology.com

Received: July 26, 2024.
Accepted: March 28, 2025.
Early view: April 10, 2025.

https://doi.org/10.3324/haematol.2024.286208

SUPPLEMENTARY APPENDIX

Supplementary Methods

Study design and participants

Inclusion criteria for the study (in addition to those listed in the main article) comprised:

- Eligible patients had de novo or transformed R/R diffuse large B-cell lymphoma for which no curative therapy was available, or indolent lymphoma (marginal zone, follicular, or mantle cell) that was R/R to standard approved therapies.
- Adequate bone marrow function, including absolute neutrophil count ≥1,000/mm^3 (≥1.0 x 10^9/L), platelets ≥50,000/mm^3 (≥50 x 10^9/L), and hemoglobin ≥8 g/dL (≥80 g/L).
- Adequate renal function, including serum creatinine ≤1.5 x upper limit of normal (ULN) or estimated creatinine clearance ≥60 mL/min (calculated using the method standard for the institution).
- Adequate liver Function, including total serum bilirubin ≤1.5 x ULN (≤3.0 x ULN if the patient has documented Gilbert syndrome), aspartate and alanine transaminase ≤3.0 x ULN (≤5.0 x ULN if there is liver involvement secondary to tumor), and alkaline phosphatase ≤2.5 x ULN (≤5.0 x ULN if bone or liver metastasis).
- QTcF interval of ≤480 msec (based upon mean value from triplicate ECGs).
- Resolved acute effects of any prior therapy to baseline severity or grade ≤1 National Cancer Institute Common Terminology Criteria for Adverse Events version 4.03 (NCI CTCAE v.4.03) except for adverse events (AE) not constituting a safety risk by investigator judgment.
- Available archival (or fresh) metastatic biopsy sample prior to study entry.
- Serum pregnancy test (for females of childbearing potential) negative at screening.
- Use of a highly effective method of contraception throughout the study and for ≥90 days after the last dose of assigned treatment for male and female patients of childbearing potential.
- Willingness and ability to comply with scheduled visits, treatment plans, laboratory, tests, and other procedures.

Patients with any of the following characteristics/conditions/treatments were excluded from the study:

- Known symptomatic CNS metastases or leptomeningeal disease requiring steroids. Patients with previously diagnosed brain metastases were eligible if they had completed treatment and

recovered from the acute effects of radiation therapy or surgery prior to study entry, had discontinued corticosteroid treatment for these metastases, were clinically stable off anticonvulsants for ≥4 weeks, and were neurologically stable before enrollment.

- History of (non-infectious) pneumonitis that required steroids or current pneumonitis.
- High-grade lymphoma (including Burkitts lymphoma, lymphoblastic lymphoma, Richter's transformation), chronic lymphocytic leukemia, or plasma cell leukemia.
- Previous high-dose chemotherapy requiring allogeneic stem cell rescue.
- Prior radiotherapy ≤2 weeks before study treatment initiation. Participants must have recovered from all radiation-related toxicities, with no requirement for corticosteroids, and had no radiation pneumonitis. A 1-week washout was permitted for palliative radiation (≤2 weeks of radiotherapy) to non-CNS disease.
- Prior treatment with any anti-CD47 or anti-SIRPα agent.
- Systemic anti-cancer therapy ≤4 weeks before starting study treatment (6 weeks for mitomycin C or nitrosoureas). If systemic anti-cancer therapy was given within 4 weeks, the patient could be included if 4–5 times the elimination half-life of the drug had passed.
- Intolerance to or previously had a severe allergic or anaphylactic reaction to antibodies or infused therapeutic proteins, or previously had a severe allergic or anaphylactic reaction to any of the substances included in the study drug (including excipients); discontinued treatment due to a grade 3 or higher immune-related AE from prior therapy with an anti-PD-1, anti-PD-L1, or anti PD-L2 agent or with an agent aiming to modulate another immune cell target (e.g. CTLA-1, OX40, 41BB, etc).
- Any experimental antibodies or live vaccines ≤28 days prior to the first dose of study drug. Examples of live vaccines include: measles, mumps, rubella, varicella/zoster, yellow fever, rabies, Bacillus Calmette–Guérin, and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and were allowed; however, intranasal influenza vaccines (e.g., FluMist®) are live attenuated vaccines and were not allowed.
- Current active therapy for the primary diagnosis.
- Blood product transfusions within 14 days of Cycle 1 Day 1.

- History of or active autoimmune disorders (including Crohn's Disease, rheumatoid arthritis, scleroderma, systemic lupus erythematosus, Grave's disease) and other conditions that compromise or impair the immune system (with the exception of hypogammaglobulinemia).
- History of autoimmune hemolytic anemia or autoimmune thrombocytopenia.
- Active, uncontrolled, clinically significant bacterial, fungal, or viral infection, including hepatitis B, hepatitis C, known human immunodeficiency virus or acquired immunodeficiency syndrome-related illness.
- Active graft versus host disease or ongoing immunosuppression for GVHD.
- Any of the following in the previous 12 months: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, poorly controlled hypertension, cerebrovascular accident, transient ischemic attack, deep venous thrombosis, arterial thrombosis, symptomatic pulmonary embolism, or any other significant thromboembolism; gastrointestinal perforation in the previous 6 months; any major surgery within 28 days prior to enrollment.
- Current active treatment in another interventional therapeutic clinical study.
- Diagnosis of any other malignancy ≤3 years prior to enrollment except for adequately treated non-melanomatous skin cancer, or carcinoma in situ (e.g., breast carcinoma, cervical cancer in situ) that had undergone potentially curative therapy.
- Other severe acute or chronic medical or psychiatric condition, including recent (within the past year) or active suicidal ideation or behavior, or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the patient inappropriate for entry into this study.
- Investigational site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the Investigator, or Sponsor employees directly involved in the conduct of the study.

The research was conducted in accordance with all relevant local/national legal and regulatory requirements, as well as the principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects and the Declaration of Helsinki. All research was approved by

the relevant institutional review boards (IRBs; Salus IRB for START Midwest, Western IRB for the University of Colorado Hospital, Dana-Faber Cancer Institute IRB for the Massachusetts General Hospital Cancer Center, and Seoul National University Hospital IRB).

Study outcomes

DLT was defined as any of the following (graded according to National Cancer Institute Common Terminology Criteria for Adverse Events version 4.032320): grade 4 neutropenia lasting >7 days; febrile neutropenia (grade ≥3 neutropenia and a single body temperature reading of >38.3□C or sustained temperature of ≥38□C for >1 hour); grade ≥3 neutropenia with infection; grade 3 thrombocytopenia with clinically significant bleeding; grade 4 thrombocytopenia; grade ≥3 toxicities, except those that had not been maximally treated; delay of ≥2 week-delays in the second treatment cycle due to persisting toxicities attributable to evorpacept; or discontinuation of any agent due to an adverse event.

Study treatments

Evorpacept was administered weekly as an intravenous (IV) infusion over approximately 60 minutes on an outpatient basis. Patients were observed in the clinic for ≥2 hours after the evorpacept infusion on cycle 1 day 1 and as clinically indicated thereafter. No premedication was required. The weekly schedule ensured that evorpacept exposure was maximized, with full CD47 receptor target occupancy, within the rituximab dosing interval. As the maximum tolerated dose was not reached in the single-agent phase and linear PK was demonstrated with ≥3 mg/kg QW, the combination-therapy phase doses and schedules were based on the optimal biological dose (OBD, i.e., the lowest dose producing a pharmacodynamic effect consistent with the proposed mechanism with acceptable toxicity), with additional doses/schedules permitted; no dosing schedule exceeded the maximum administered dose in the single-agent phase (10 mg/kg once weekly and 30 mg/kg once every other week).

Rituximab 375 mg/m^2 was administered as an IV infusion once weekly for 4 doses followed by once monthly for 8 doses. Rituximab was initiated at a rate of 50 mg/hour and increased by 50 mg/hour increments every 30 minutes, to a maximum of 400 mg/hour in the absence of infusion toxicity. If the first infusion of rituximab was tolerated, subsequent infusions could be started at a rate of 100

mg/hour and increased by 100 mg/hour increments at 30-minute intervals, to a maximum of 400 mg/hour in the absence of toxicity. On administration days when evorpacept and rituximab dosing schedules coincided, rituximab administration was started ~30 minutes after evorpacept therapy finished. On such days, in the event of a missed dose of evorpacept due to toxicity, rituximab could be administered 24 hours after the missed dose.

No additional anti-tumor treatments, live vaccines, or chronic systemic corticosteroids were permitted during the study. Palliative care and supportive care for NHL symptoms were allowed at the investigator's discretion. Palliative radiotherapy was permitted for the treatment of painful bony lesions providing the lesions were known at the time of study entry and the Investigator clearly indicated that the need for palliative radiotherapy was not indicative of disease progression. While hematopoietic growth factors (e.g., erythropoietin) were prohibited during the first treatment cycle, patients could take them from cycle 2 onward. Primary prophylaxis of diarrhea, nausea and vomiting was permitted at the Investigator's discretion.

Assessments

AE were assessed and reported at each study visit (with severity graded by the NCI CTCAE v.4.03), and patients were followed up for up to 28 days after the last study treatment administration or until all drug-related toxicities had resolved, whichever was later. Tumor assessments were performed approximately every 8 weeks from the start of study treatment until disease progression, death, or permanent discontinuation of study treatment. Imaging included chest, abdomen, and pelvis CT or MRI scans; brain CT or MRI scan for patients with known or suspected brain metastases; bone scan and/or bone x-rays for patients with known or suspected bone metastases. PET scans and bone marrow evaluation may also be performed. Hematology and blood chemistry parameters were assessed on days 1, 8 and 15 of cycle 1, days 1 and 15 of cycle 2, day 1 of all subsequent cycles, and at the end of treatment. Coagulation was evaluated on day 1 of cycles 1 and 2, and at the end of treatment.

Analysis of PK, PD, and immunogenicity

Evorpacept serum concentrations for evaluation of PK and the presence of anti-evorpacept antibodies

were analyzed using validated enzyme-linked immunosorbent assays. Recombinant CD47 protein was used to capture evorpacept present in serum, which was detected by goat anti-human immunoglobulin (H+L) horseradish peroxidase conjugate in the presence of the substrate 3, 3', 5, 5'-tetramethylbenzidine. The assays were performed at Syneos Health (Princeton, NJ 08540). PK parameters (including maximum concentration, area under the concentration time curve to infinity, clearance, and volume of distribution) were determined from the respective concentration–time data using standard noncompartmental methods. Drug concentrations of evorpacept and noncompartmental PK parameters were summarized by dose.

Complete CD47 target occupancy in peripheral blood T lymphocytes and erythrocytes was defined as ≥85% during validation of the flow cytometry assay because the dynamic equilibrium between evorpacept-bound CD47 and free CD47 varies according to the processing method and time between blood collection and analysis. CD47 target occupancy was measured by two fit-for-purpose, validated flow cytometry assays on T lymphocytes and erythrocytes in peripheral whole blood. A fluorescently labeled CD47 antibody (clone B6H12) was used to quantify the free CD47 binding sites in the presence of evorpacept. The assays were performed at Covance Central Laboratory Services (Indianapolis, IN 46214).

Levels of immune-related biomarkers (e.g., $CD8^+$, and $CD68^+$ and $CD163^+$ immune cell populations) on tumor biopsy tissue were measured by immunohistochemistry (IHC) on formalin-fixed, paraffin-embedded tumor biopsy samples. Single-stain IHC with 3,3'-diaminobenzidine chromogen was performed using the following IHC primary antibody: 1) CD8, mouse clone C8/144B (Dako, Santa Clara, CA); 2) CD163, mouse clone 10D6 (Leica, Deer Park, IL); and 3) CD68, mouse clone KP1 (Dako, Santa Clara, CA). Percent positive values for CD8, CD68, and CD163 were in regions of interest: 1) 'intra-tumoral' as tumor plus intervening stroma; and 2) peri-tumoral region (not reported). Percent positive values for CD8, CD68, and CD163 were obtained by image analysis using ImageScope software (Aperio) or, in limited cases, using a pathologist's visual score. All tumor IHC staining, image analysis, and pathologist review and scoring were performed at Mosaic Laboratory (Lake Forest, CA 92630). The percentage of positive cells/mm^2 was determined for the regions of interest using a cytonuclear algorithm (Indica Labs, Corrales, NM, USA).

Statistical analysis

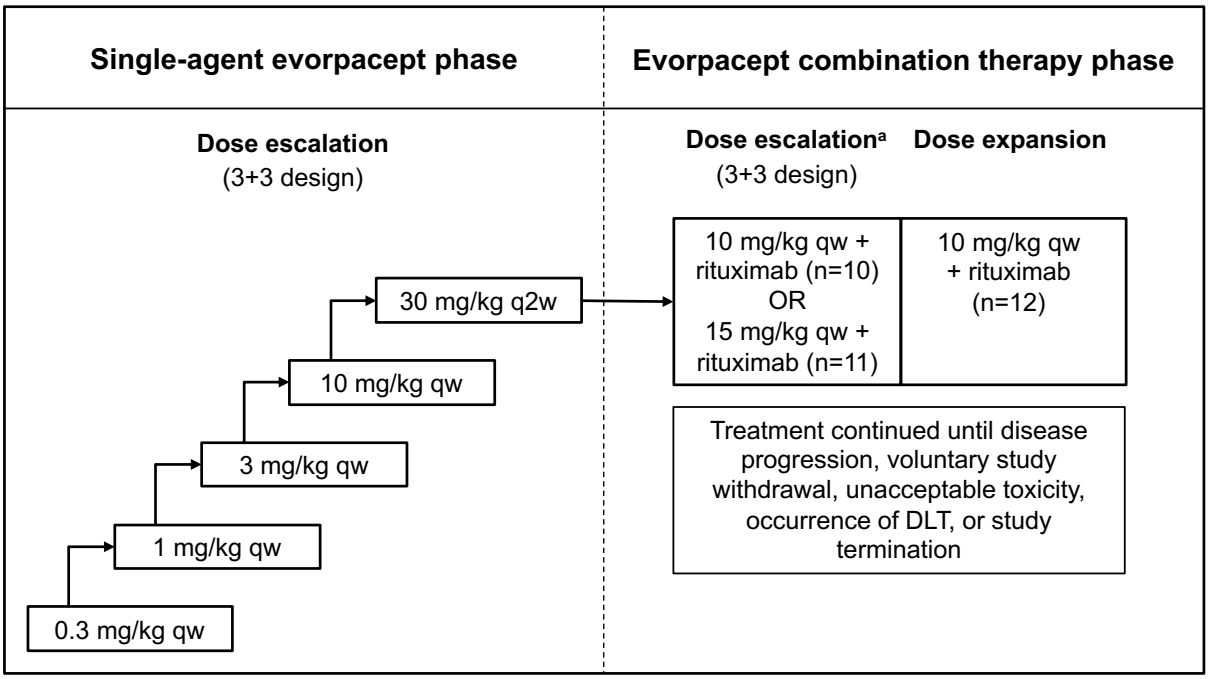
The sample size in the combination-therapy phase depended on the safety profile observed in the single-agent phase and determined the number of patients at each dose level and the number of dose levels investigated. In the combination-therapy dose-escalation cohort, once the MTD or MAD was reached, an additional 9 patients were enrolled at that dose level for a total of approximately 15 patients to confirm safety. In a 15-patient cohort, a toxicity that occurred in 10% of patients had a 79% chance of being observed in ≥1 patient. When the MTD or OBD had been determined, approximately 20–40 patients were enrolled to further evaluate safety and anti-tumor activity with evorpacept plus rituximab in the dose-expansion phase. With 20 patients in the expansion cohort, there was an 88% chance of detecting a toxic effect in 10% of patients, and a >96% chance of identifying responders if the true ORR was >15%.Continuous and categorical data were summarized using descriptive statistics and binary data using percentage rates with 95% confidence intervals (CI). PK parameters were assessed using conventional non-compartmental methods and the WinNonlin® software package (Phoenix® WinNonlin® Professional, version 8.3; Certara, Princeton, NJ, USA).

Supplementary Table S1. Summary of treatment-emergent adverse events irrespective of causality, by system organ class and preferred term (occurring in >10% of patients overall).

No. of patients (%)	**Evorpacept 10 mg/kg (n=22)**	**Evorpacept 15 mg/kg (n=11)**	**Total (N=33)**
Overall total (any treatment-emergent adverse event, irrespective of causality)	19 (86.4)	9 (81.8)	28 (84.8)
Skin and subcutaneous disorders	10 (45.5)	4 (36.4)	14 (42.4)
Rash	7 (31.8)	2 (18.2)	9 (27.3)
Infections and infestations	10 (45.5)	6 (54.5)	16 (48.5)
Nasopharyngitis	4 (18.2)	2 (18.2)	6 (18.2)
General disorders and administration-site conditions	14 (63.6)	5 (45.5)	19 (57.6)
Fatigue	5 (22.7)	3 (27.3)	8 (24.2)
Pyrexia	6 (27.3)	2 (18.2)	8 (24.2)
Injury, poisoning, and procedural complications	6 (27.3)	5 (45.5)	11 (33.3)
Infusion-related reaction	5 (22.7)	5 (45.5)	10 (30.3)
Investigations	8 (36.4)	3 (27.3)	11 (33.3)
ALT increased	3 (13.6)	1 (9.1)	4 (12.1)
Neutrophil count decreased	2 (9.1)	2 (18.2)	4 (12.1)
Respiratory, thoracic, and mediastinal disorders	5 (22.7)	3 (27.3)	8 (24.2)
Dyspnea	2 (9.1)	2 (18.2)	4 (12.1)
Blood and lymphatic system disorders	7 (31.8)	1 (9.1)	8 (24.2)
Anemia	5 (22.7)	0	5 (15.2)
Musculoskeletal and connective tissue disorders	7 (31.8)	3 (27.3)	10 (30.3)
Myalgia	4 (18.2)	0	4 (12.1)
Metabolism and nutrition disorders	6 (27.3)	1 (9.1)	7 (21.2)
Decreased appetite	4 (18.2)	0	4 (12.1)

ALT: alanine aminotransferase.

Supplementary Figure S1. Study schema.



[a]Evorpacept was initiated at one dose level below single-agent MTD/MAD, with the observed single-agent evorpacept DLT profile and known safety profile of the combination agent considered.

DLT: dose-limiting toxicity; MAD: maximum administered dose; MTD: maximum tolerated dose; q2w: once every other week; qw: once weekly.