Supplementary Material

A Phase I Study of Prophylactic Anakinra to Mitigate Immune Cell Associated Neurotoxicity Syndrome in Patients with Large B-Cell Lymphoma

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Eligibility Criteria

All inclusion and exclusion criteria required for eligibility for this phase I study are detailed below.

Inclusion Criteria

1. Relapsed or refractory diffuse large B-cell lymphoma, primary mediastinal B-cell lymphoma, transformed follicular lymphoma, or high-grade B-cell lymphoma, with at least 2 prior lines of systemic therapy
2. Planned standard-of-care therapy with axicabtagene ciloleucel
3. ≥ 18 years of age
4. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1
5. Measurable disease of ≥ 1.5 cm
6. At the time of planned axi-cel therapy, at least 2 weeks or 5 half-lives, whichever is shorter, must have elapsed since any prior systemic therapy, except for systemic immune checkpoint inhibitory or immune-stimulatory therapy (e.g., ipilimumab, nivolumab, pembrolizumab, atezolizumab, OX40 agonists, 4-1BB agonists), which could be administered within this period, and at the time of planned leukapheresis for manufacture of axi-cel, at least 3 half-lives must have elapsed from any prior systemic immune checkpoint inhibitory or immune-stimulatory therapy

7. Toxicities due to prior therapy were required to be stable and recovered to grade 1 or lower (except for clinically non-significant toxicities such as alopecia)
8. Absolute neutrophil count of $\geq 1.0\times10^9$/L
9. Platelet count of $\geq 60\times10^9$/L
10. Creatinine clearance (as estimated by Cockcroft-Gault) ≥ 45 mL/min
11. Serum alanine transaminase and aspartate transaminase ≤ 2.5 upper limit of normal
12. Total bilirubin ≤ 1.5 mg/dL, except in patients with Gilbert syndrome
13. Cardiac ejection fraction ≥ 50% with no evidence of pericardial effusion
14. Baseline oxygen saturation > 92% on room air
15. No evidence, suspicion, and/or history of lymphoma involving the central nervous system (CNS)
16. Female patients of childbearing potential were required to have a negative serum or urine pregnancy test (female patients who have undergone surgical sterilization or who have been postmenopausal for at least 2 years are not considered to be of childbearing potential)

Exclusion Criteria

Patients were excluded from participating in the trial if any of the following criteria were met:

1. History of malignancy other than nonmelanoma skin cancer or carcinoma in situ (e.g., of the cervix, bladder, breast) unless disease free for at least 3 years
2. History of Richter transformation of chronic lymphocytic leukemia
3. Autologous stem cell transplant within 6 weeks of planned axi-cel infusion
4. History of allogeneic stem cell transplant

5. Prior CD19-targeted therapy
6. Prior chimeric antigen receptor therapy or other genetically modified T-cell therapy
7. Presence of fungal, bacterial, viral, or other infection that was uncontrolled or required intravenous antimicrobials for management; simple urinary tract infection and uncomplicated bacterial pharyngitis were permitted if the infection was responding to active treatment and after consultation with the principal investigator
8. Known history of infection with HIV or hepatitis B (HBsAg positive) or hepatitis C virus (anti-HCV positive); a history of hepatitis B or hepatitis C was permitted if the viral load was undetectable per quantitative polymerase chain reaction and/or nucleic acid testing
9. Known history of tuberculosis; a negative QuantiFERON or purified protein derivative (PPD) up to 2 months before the start of anakinra was enough if there were no specific risk factors
10. Presence of any indwelling line or drain (e.g., percutaneous nephrostomy tube, indwelling Foley catheter, biliary drain, or pleural/peritoneal/pericardial catheter); dedicated central venous access catheters such as a port-a-cath or Hickman catheter were permitted
11. Patients with detectable cerebrospinal fluid malignant cells or brain metastases, or with a history of CNS lymphoma, cerebrospinal fluid malignant cells, or brain metastases
12. History or presence of CNS disorder such as seizure disorder, cerebrovascular ischemia/hemorrhage, dementia, cerebellar disease, or any autoimmune disease with CNS involvement
13. Cardiac atrial or cardiac ventricular lymphoma involvement

14. History of myocardial infarction, cardiac angioplasty or stenting, unstable angina, or other clinically significant cardiac disease within 12 months of enrollment
15. Requirement for urgent therapy due to tumor mass effects such as bowel obstruction or blood vessel compression
16. Primary immunodeficiency
17. History of autoimmune disease (e.g. Crohn's disease, rheumatoid arthritis, systemic lupus) resulting in end organ injury or requiring systemic immunosuppression/systemic disease-modifying agents within the last 2 years
18. History of symptomatic deep vein thrombosis or pulmonary embolism within 6 months of enrollment
19. Any medical condition likely to interfere with assessment of safety or efficacy of study treatment
20. History of severe immediate hypersensitivity reaction to any of the agents used in this study, including *Escherichia coli*–derived proteins
21. Live vaccine ≤ 6 weeks prior to planned start of conditioning regimen
22. Women of child-bearing potential who would be pregnant or breastfeeding at the time of enrollment because of the potentially dangerous effects of the preparative chemotherapy on the fetus or infant
23. Patients of any gender who were not willing to practice birth control from the time of consent through 6 months after the completion of anakinra injections
24. In the investigator's judgment, the patient was unlikely to complete all protocol-required study visits or procedures, including follow-up visits, or comply with the study requirements for participation

DLT definition

Dose-limiting toxicity (DLT) was defined as any of the following events related to sequenced therapy (that is, related to the addition of anakinra to the approved axi-cel treatment regimen) with onset within the first 28 days following the start of anakinra and axi-cel infusion:

1. Grade 4 neutropenia lasting longer than 21 days from the day of axi-cel infusion
2. Grade 4 thrombocytopenia lasting longer than 28 days from the day of axi-cel infusion
3. Any sequenced therapy–related adverse event requiring intubation, including grade 4 encephalopathy requiring intubation for airway protection
4. Any sequenced therapy–related grade 5 event
5. All other clinically significant grade 3 toxicities lasting more than 3 days and all grade 4 toxicities, except for the following conditions, which were not considered DLTs:
    - Encephalopathy that resolved to at worst grade 1 within 2 weeks and to baseline within 4 weeks
    - Grade 3 fever
    - Myelosuppression (includes bleeding in the setting of platelet count $< 50 \times 10^9$/L and documented bacterial infections in the setting of neutropenia), defined as lymphopenia, decreased hemoglobin, neutropenia, and thrombocytopenia unless neutropenia and thrombocytopenia met the DLT definitions above

- Immediate hypersensitivity reactions occurring within 2 hours of axi-cel infusion or anakinra injection that were reversible to grade 2 or less within 24 hours of administration of standard therapy
- Renal toxicity requiring dialysis for ≤ 7 days
- Tumor lysis syndrome, including associated manifestations attributable to tumor lysis syndrome (e.g., electrolyte abnormalities, renal function, hyperuricemia)
- Grade 3 transaminase, alkaline phosphatase, bilirubin, or other liver function marker elevation that resolved to grade 2 or less within 14 days
- Grade 4 transient serum hepatic enzyme abnormalities that resolved to grade 3 or less within < 72 hours
- Grade 3 or 4 hypogammaglobulinemia
- Grade 3 nausea or anorexia

If grade 3 or 4 adverse events representing changes in vital signs and/or neurological status were not due to CRS or ICANS but to one of the exceptions above, the events were not considered a DLT.

Statistical considerations for contemporaneous matched cohort

We matched the 20 patients in the pilot study with those from a contemporaneous cohort that received axi-cel but not anakinra. There were 36 total patients in the contemporaneous cohort. For each patient in the pilot study, we first looked for all contemporaneous cohort subjects who were a perfect match by gender, refractory status, and International Prognostic Index (IPI). If more than one patient remained in the contemporaneous cohort, we calculated standardized differences in age and total metabolic tumor volume. The contemporaneous cohort patient with the smallest standardized differences was then selected as the match. If there were no perfect matches on all three of gender, refractory status, and IPI, we looked for perfect matches by refractory status and IPI. In this case, we then looked at standardized differences in age, total metabolic tumor volume, and IPI to break ties.

Supplementary Table 1. Baseline characteristics of contemporaneous matched cohort

Characteristic	Number (%), median [range]
DLBCL/HGBCL	16 (80)
Age, years	56 [21-79]
Age > 60 years	8 (40)
Male	14 (70)
ECOG performance status ≥ 1	4 (20)
Ann Arbor stage III-IV	15 (75)
Extra-nodal sites > 1	8 (40)
International Prognostic Index score ≥ 3	11 (55)
Absolute neutrophil count, 10^9/L	4 [0.95-24.4]
Absolute lymphocyte count, 10^9/L	0.5 [0.02-3.1]
Absolute monocyte count, 10^9/L	0.5 [0.03-3.3]
Hemoglobin, g/dL	11.4 [7.9-14.3]
Platelet count, 10^9/L	172 [98-529]
C-reactive protein, mg/L	27 [0.3-172]
Ferritin, mg/L	633 [17-4088]
Lactate dehydrogenase, U/L	277 [126-1716]
Lactate dehydrogenase > UNL	15 (75)
Creatinine clearance, mL/min	92 [24-129]
Previous therapies	3 [2-7]
Bridging therapy use	11 (55)
Bridging: Chemotherapy	5 (20)
Radiation therapy	0 (0)
Biological therapy	6 (30)
Refractory disease	16 (80)
Previous autologous SCT	2 (10)
Previous allogeneic SCT	1 (5)
Total metabolic tumor volume, mL	25 [0.1-2037]

DLBCL, diffuse large B-cell lymphoma; HGBCL, high-grade B-cell lymphoma; SCT, stem cell transplant; UNL, upper normal limit

Supplementary Table 2. All adverse events noted on study

	Dose level 1, 100 mg daily (n=10), number (%)		Dose level 2, 100 mg twice a day (n=10), number (%)		All (n=20), number (%)	
Adverse Event	**Grade 1-2**	**Grade 3-4**	**Grade 1-2**	**Grade 3-4**	**Grade 1-2**	**Grade 3-4**
Neutropenia	1 (10)	5 (50)	0 (0)	8 (80)	1 (5)	13 (65)
Thrombocytopenia	1 (10)	5 (50)	3 (30)	4 (40)	4 (20)	9 (45)
Anemia	3 (30)	4 (40)	3 (30)	5 (50)	6 (30)	9 (45)
Infection	1 (10)	3 (30)	2 (20)	0 (0)	3 (15)	3 (15)
Hypertension	0 (0)	2 (20)	0 (0)	1 (10)	0 (0)	3 (15)
Hypoxemia	2 (20)	1 (10)	1 (10)	1 (10)	3 (15)	2 (10)
Diarrhea	2 (20)	1 (10)	3 (30)	0 (0)	5 (25)	1 (5)
Hypotension	5 (50)	1 (10)	1 (10)	0 (0)	6 (30)	1 (5)
Hypofibrinogenemia	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	1 (5)
Hematuria	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	1 (5)
Hypernatremia	0 (0)	1 (10)	0 (0)	0 (0)	0 (0)	1 (5)
Fever	9 (90)	0 (0)	7 (70)	0 (0)	16 (80)	0 (0)
Nausea	5 (50)	0 (0)	8 (80)	0 (0)	13 (65)	0 (0)
Fatigue	5 (50)	0 (0)	7 (70)	1 (10)	12 (60)	1 (5)
Headache	5 (50)	0 (0)	5 (50)	1 (10)	10 (50)	1 (5)
Anorexia	5 (50)	0 (0)	4 (40)	0 (0)	9 (45)	0 (0)
Pain	3 (30)	0 (0)	6 (60)	0 (0)	9 (45)	0 (0)
Sinus tachycardia	4 (40)	0 (0)	3 (30)	0 (0)	7 (35)	0 (0)
ALT increased	3 (30)	0 (0)	3 (30)	0 (0)	6 (30)	0 (0)
Hyponatremia	1 (10)	0 (0)	4 (40)	0 (0)	5 (25)	0 (0)
AST increased	3 (30)	0 (0)	1 (10)	0 (0)	4 (20)	0 (0)
Arrhythmia	3 (30)	0 (0)	1 (10)	0 (0)	4 (20)	0 (0)
Edema limbs	1 (10)	0 (0)	3 (30)	0 (0)	4 (20)	0 (0)
Constipation	1 (10)	0 (0)	3 (30)	0 (0)	4 (20)	0 (0)
Insomnia	2 (20)	0 (0)	2 (20)	0 (0)	4 (20)	0 (0)
Skin rash	2 (20)	0 (0)	2 (20)	0 (0)	4 (20)	0 (0)
Serosal effusion	3 (30)	0 (0)	1 (10)	0 (0)	4 (20)	0 (0)
Cough	1 (10)	0 (0)	2 (20)	0 (0)	3 (15)	0 (0)
Creatinine increased	1 (10)	0 (0)	2 (20)	0 (0)	3 (15)	0 (0)
Tremors	2 (20)	0 (0)	1 (10)	0 (0)	3 (15)	0 (0)
CMV infection	2 (20)	0 (0)	0 (0)	0 (0)	2 (10)	0 (0)
Bilirubin increased	1 (10)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)
Agitation	1 (10)	0 (0)	0 (0)	0 (0)	1 (5)	0 (0)

ALT, alanine transferase; AST, aspartate transferase; CMV, cytomegalovirus

Supplementary Table 3. Toxicity and management

Characteristic		Number (% [95%CI]), median [range]			
		Dose level 1, 100 mg daily (n=10)	Dose level 2, 100 mg twice daily (n=10)	Total (n=20)	Matched cohort (n=20)
CRS	Any grade	10 (100 [69.2, 100])	9 (90 [55.5, 99.7])	19 (95 [75.1, 99.9])	19 (95 [75.1, 99.9])
	Grade 2-4	5 (50 [18.7, 81.3])	3 (30 [6.7, 65.2])	8 (40 [19.1, 63.9])	10 (50 [27.2, 72.8])
	Grade 3-4	1 (10 [0.3, 44.5])	0 (0 [0, 30.8])	1 (5 [0.1, 24.9])	1 (5 [0.1, 24.9])
	Onset, day	2 [1-7]	4 [3-8]	3 [1-8]	4 [0-10]
	Duration, days	5 [1-10]	4 [2-9]	5 [2-9]	3 [2-9]
ICANS	Any grade	4 (40 [12.2, 73.8])	3 (30 [6.7, 65.2])	7 (35 [15.4, 59.2])	12 (60 [36.1, 80.9])
	Grade 3-4	2 (20 [2.5, 55.6])	2 (20 [2.5, 55.6])	4 (20 [5.7, 43.7])	6 (30 [11.9, 54.3])
	Onset, day	7 [3-8]	8 [5-9]	7 [3-9]	5 [1-14]
	Duration, days	1 [1-16]	4 [2-6]	2 [2-17]	3 [1-24]
Patients requiring corticosteroids		4 (40)	3 (30)	7 (35)	11 (55)
Dexamethasone	Cumulative dose, mg	40 [10-1728]	100 [20-630]	100 [10-1728]	112 [4-445]
	Start, day	7 [3-8]	8 [4-9]	7 [3-9]	5 [1-11]
	Duration, days	3 [1-20]	5 [2-12]	4 [1-20]	6 [1-20]
Tocilizumab	Patients requiring tocilizumab	7 (70)	5 (50)	12 (60)	11 (55)
	Doses	2 [1-3]	1 [1-2]	2 [1-3]	2 [1-3]
	Start, day	5 [3-6]	6 [4-9]	5 [3-9]	5 [1-11]
	Duration, days	2 [1-5]	1 [1-2]	1 [1-5]	2 [1-3]

CRS, cytokine release syndrome; ICANS, immune effector cell–associated neurotoxicity syndrome