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Phase 2 pilot study to evaluate efficacy and safety of anakinra to prevent CD19-targeted CAR-T cell-related cytokine release syndrome (CRS) and neurotoxicity in patients with B cell lymphoma

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Protocol Synopsis

Protocol Number: 10373 (RG1006866)	
Title of Study: Phase 2 pilot study to evaluate efficacy and safety of anakinra to prevent CD19-targeted CAR-T cell-related cytokine release syndrome (CRS) and neurotoxicity in patients with B-cell lymphoma (B-NHL)	
Financial Support: Sobi	**Phase of Development:** 2
IND Number: 147757	

Study Objectives:

Primary:

Decrease the incidence of CRS after treatment with lisocabtagene ciloleucel (liso-cel; Breyanzi®)

Secondary:

1. Decrease the severity of CRS.
2. Decrease the incidence and severity of neurotoxicity.
3. Decrease the rate and duration of hospitalization.
4. Decrease corticosteroid usage.
5. Evaluate the safety of anakinra after liso-cel infusion.
6. Evaluate the effect of anakinra on liso-cel efficacy for treatment of B-NHL

Exploratory:

1. To assess the pharmacokinetics of anakinra in B-NHL patients undergoing CAR T-cell therapy
2. To evaluate the effects of anakinra on the immune system (e.g, CAR T cells, other immune cells, serum cytokine concentrations)

Study Purpose and Rationale:

T cells engineered with CD19-targeted chimeric antigen receptors (CD19 CAR T cells) have shown promising efficacy in patients with relapsed or refractory B cell malignancies, including B cell non-Hodgkin lymphomas (B-NHL), but this approach is limited by significant toxicities: cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Severe toxicities have been reported across all FDA-approved CD19 CAR T cell products for B-NHL[1-7] (grade ≥3 CRS, 2-22%; grade ≥3 ICANS, 8-37%). Murine models have shown monocyte-derived IL-1 and IL-6 are required for the development of CRS and ICANS after CD19 CAR T-cell therapy, and that these toxicities could be mitigated using anakinra (Kineret®), a recombinant, nonglycosylated form of the human interleukin-1 receptor antagonist. Anakinra is FDA-approved for the treatment of rheumatoid arthritis and neonatal-onset multisystem inflammatory disease. Early clinical data suggest anakinra might be efficacious in humans to treat severe CRS and ICANS after CAR T cell therapy, but there are no data to date regarding its use in the prophylactic setting. This pilot study aims at evaluating the feasibility and efficacy of the administration of anakinra to prevent CRS and ICANS in LBCL patients treated with the FDA-approved CD19 CAR T-cell product liso-cel.

Study Population:

Inclusion Criteria:

1) Subjects must be 18 years of age or older.
2) Karnofsky performance status of ≥ 60%.
3) Patients with B-NHL eligible for treatment with liso-cel. Patients treated with non-conforming (out-of-specification) liso-cel may remain on study.
4) Negative serum pregnancy test within 2 weeks of enrollment for women of childbearing potential, defined as those who have not been surgically sterilized or who have not been free of menses for at least 1 year.
5) Fertile male and female subjects must be willing to use an effective contraceptive method before, during, and for at least 4 months after the last dose of anakinra.
6) Ability to understand and provide informed consent.

Exclusion Criteria:

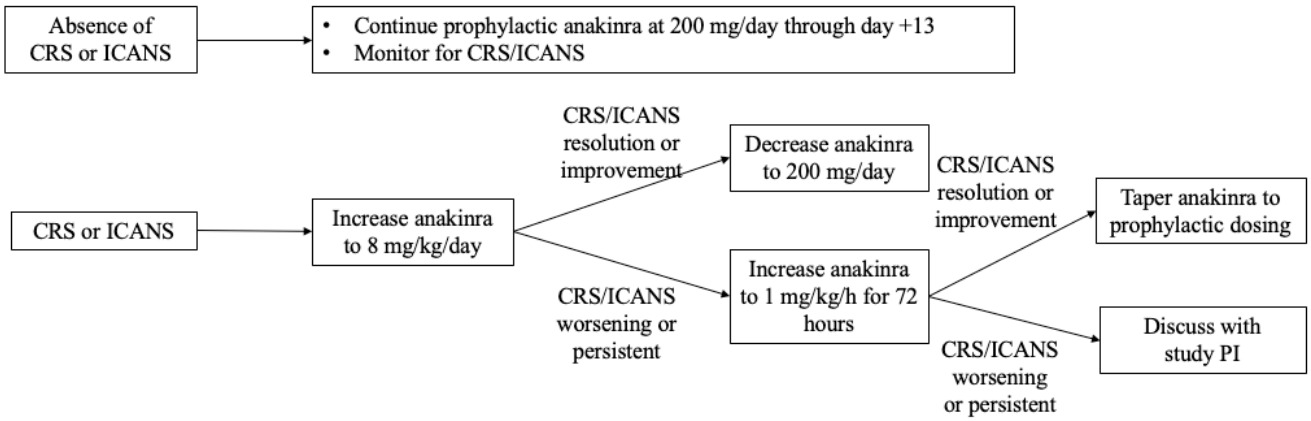
1) Subjects requiring ongoing daily corticosteroid therapy at a dose of >15 mg of prednisone per day (or equivalent). Pulsed corticosteroid use for disease control is acceptable.
2) Active autoimmune disease requiring immunosuppressive therapy is excluded unless discussed with the PI.
3) Known hypersensitivity to E coli-derived proteins, anakinra, or to any component of the product.
4) Major organ dysfunction defined as:
   a. Serum creatinine > 2.5 mg/dL
   b. Significant hepatic dysfunction (Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) > 5x upper limit of normal; bilirubin > 3.0 mg/dL) unless due to malignancy or Gilbert's syndrome in the opinion of the PI or designee.
   c. Subjects with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing. Those with an FEV1 of < 50 % of predicted or DLCO (corrected) < 40% will be excluded.
   d. Significant cardiovascular abnormalities as defined by any one of the following: NYHA class III or IV congestive heart failure, clinically significant hypotension, uncontrolled symptomatic coronary artery disease, or a documented ejection fraction of <35%.
5) Uncontrolled serious and active infection.

Test Product, Dose, and Mode of Administration:
In the first 15 treated patients, anakinra 200 mg/day was administered as two simultaneous 100 mg subcutaneous injections daily (approximately every 24 hours) for 14 days, with the first administration approximately 2-4 hours prior to liso-cel infusion (day 0 through day 13).
Prophylactic dosing:
In the expansion cohort (n=10), anakinra will be administered first at the prophylactic dosing of 200 mg once daily intravenously (approximately every 24 hours) for 14 days, with the first administration approximately 2-4 hours prior to liso-cel infusion (day 0 through day 13).

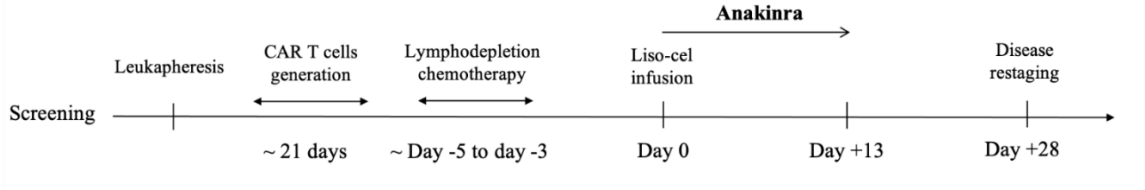
Anakinra up-titration:
Step 1 (8 mg/kg/day): In patients who develop CRS or ICANS during anakinra prophylaxis at 200 mg/day, anakinra will be increased to 8 mg/kg/day. The step 1 anakinra dose will be calculated using the participant's actual or ideal body weight as per PI discretion. The total daily dose will be divided over three doses in 24 hours, each dose will be rounded to the nearest 100 mg, diluted in NaCl 0.9%, and given IV over approximately 10-30 minutes. Once CRS and ICANS symptoms are improved or resolved, as evaluated by the PI or designee, anakinra will be decreased to the prophylactic dosing (200 mg/day).

Step 2 (1 mg/kg/h): In the absence of improvement or worsening of CRS or ICANS symptoms despite anakinra treatment at 8 mg/kg/day, as evaluated by the PI or designee, anakinra will be increased once to 1 mg/kg/h administered as a continuous IV infusion for 72 hours. The step 2 anakinra dose will be calculated using the participant's actual or ideal body weight as per PI discretion, rounded to the nearest 100 mg, and diluted in NaCl 0.9%. After 72 hours of anakinra at 1 mg/kg/h, anakinra will be decreased to 8 mg/kg/day. Once CRS and ICANS symptoms are improved or resolved on anakinra at 8 mg/kg/day, as evaluated by the PI or designee, anakinra will be further decreased to the prophylactic dosing.

Anakinra up-titration algorithm:



Study schema:



Safety Assessments:
Adverse events will be monitored and recorded throughout the investigative phases of the study: from the first dose of anakinra through day 28 after liso-cel infusion. Related AEs will be monitored until stabilization or resolution.

Efficacy Assessments: CRS and ICANS will be monitored and recorded throughout the investigative phases of the study: from the first dose of anakinra through day 28 after liso-cel infusion.
Statistical Methods: The efficacy of anakinra in preventing the occurrence of any grade CRS will be assessed using the Bayesian optimal phase 2 (BOP2) design with a planned interim analysis after 15 patients are treated. We will conclude that the primary endpoint is not met if $\leq$ 9 patients without CRS are observed in the first 15 patients treated with anakinra, or if $\leq$18 patients without CRS are observed in 25 patients treated with anakinra. Since this is a pilot study, the decision to include the additional 10 patients might be considered based on both the primary and secondary endpoints. **February 2023 update:** although our interim analysis did not meet the primary endpoint, anakinra prophylaxis was feasible, safe, and associated with low rates of mild CRS (8/15; 53%) and ICANS (3/15; 20%). Longitudinal analyses of the participants' temperature and CRP data suggested faster resolution of systemic inflammation compared to patients receiving liso-cel alone. Therefore, we will plan to treat 10 additional patients with the following protocol modification: change from SC to IV administration route, up-titration of the anakinra dose in case of breakthrough CRS or ICANS.
Sample size: Up to 25 patients

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1.0 BACKGROUND

This is a phase 2 pilot study to evaluate the efficacy and safety of anakinra to prevent CD19-targeted CAR-T cell-related cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome ICANS in patients with B-cell non-Hodgkin lymphoma (B-NHL). The study will be conducted in compliance with the Institutional Review Board (IRB) approved protocol, associated Federal regulations and all applicable IRB requirements.

1.1 CD19-targeted CAR T-cell therapy for relapsed or refractory B-cell NHL

Immunotherapy with T cells engineered to express a CD19-targeted chimeric antigen receptor (CD19 CAR T-cell therapy) has shown promising efficacy in patients with relapsed or refractory B cell malignancies. To date, three CD19-targeted CAR-T cell products have already been approved by the FDA for patients with R/R B-NHL: axicabtagene ciloleucel (axi-cel; Yescarta®) and tisagenlecleucel (tisa-cel, Kymriah®) for R/R large B cell lymphoma (LBCL), and brexucabtagene autoleucel (brexu-cel; Tecartus®) for R/R mantle cell lymphoma based on the pivotal clinical trials ZUMA-1[1], JULIET[3], and ZUMA-2[6], respectively. Another CD19 CAR T-cell product, lisocabtagene ciloleucel (liso-cel; Breyanzi®), was recently approved by the FDA for R/R B-NHL based on the results of the TRANSCEND NHL 001 clinical trial[7]. In this high-risk population, overall and complete responses were achieved in 52-93% and 40-67% of patients, respectively.

1.2 Significant toxicities after CD19 CAR T-cell therapy in patients with R/R B-cell NHL

The promising results of CD19 CAR-T cell therapy in R/R B-cell NHL are tempered by two potentially life-threatening toxicities: CRS and ICANS. The incidence, severity, and timing of CRS and ICANS across pivotal trials and non-trial settings are detailed in Table 1. Higher incidence of severe CRS and ICANS have been reported after axi-cel and brexu-cel treatment compared to tisa-cel and liso-cel. While the most favorable toxicity profile has been observed after liso-cel, 42% and 30% of patients treated on the TRANSCEND clinical trial still experienced any grade CRS and ICANS, respectively.

Table 1. CRS and ICANS after CD19 CAR T-cell therapy for R/R LBCL

	Axi-cel			**Brexu-cel**	**Tisa-cel**	**Liso-cel**
	ZUMA-1[1]	**Nastoupil et al [8]**	**Jacobson et al [9]**	**ZUMA-2[6]**	**JULIET[3]**	**TRANSCEND[7]**
No. of patients treated	101	275	122	60	93	269
CRS (graded according to the 2014 Lee criteria for all studies except JULIET*)						
Any grade / ≥ grade 3	**93 % / 11%**	**91% / 7%**	**93% / 16%**	**91%/15%**	**58%/22%**	**42%/2%**
Median time to onset, range	2 days (1-12)	NR	3 days (0-20)	3 days (1-13)	3 days (3-9)	5 days (1-14)
Median duration, range	8 days (NR)	NR	6 days (1-27)	10 days (1-50)	7 days (2-30)	NR
ICANS (graded according to the CTCAE 4.03)						
Any grades / ≥ grade 3	**64 % / 28%**	**69% / 31%**	**70% / 35%**	**63%/31%**	**21%/12%**	**30%/10%**
Median time to onset, range	5 days (1-17)	NR	5 days (0-34)	6 days (1-32)	6 days (1-17)	9 days (1-66)
Median duration, range	17 days (NR)	NR	7 days (1-52)	21 days (2-454)	14 (NR)	NR

Abbreviations: Axi-cel, axicabtagene ciloleucel; brexu-cel, brexucabtagene autoleucel; tisa-cel, tisagenlecleucel; liso-cel, lisocabtagene maraleucel; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; NR, not reported. *CRS graded using the PENN scale.

1.3 Current management of CRS and ICANS in patients undergoing CD19 CAR T-cell therapy

The current management of CRS and ICANS recommended in the product-specific Risk Evaluation and Mitigation Strategies[10,11] are based on the use of tocilizumab (IL-6 receptor[IL-6R]-directed monoclonal antibody) and corticosteroids, which showed efficacy in early phase I/II clinical trials of CD19 CAR T-cell therapy[12-15]. While tocilizumab can efficiently treat CRS in most patients, it may not be as effective in preventing or treating ICANS.[16] This might be due to: (i) the poor CNS penetration of tocilizumab when administered intravenously[17]; (ii) the increase in the serum concentrations of IL-6 following tocilizumab administration, possibly due to impaired IL-6R-mediated clearance of IL-6[18], and receptor-mediated endocytosis of the IL-6R upon binding to tocilizumab.[19]
Overall, the efficacy of tocilizumab and corticosteroids remain suboptimal, particularly to prevent or treat ICANS; this represents a critically unmet need to improve the toxicity profile of CD19 CAR T-cell therapies.

2.0 STUDY PURPOSE AND RATIONALE

2.1 Description of the investigational drug product anakinra

Anakinra (Kineret®) is a recombinant, nonglycosylated form of the IL-1R antagonist (IL-1Ra), which is FDA-approved for the treatment of rheumatoid arthritis and Neonatal-Onset Multisystem Inflammatory Disease (NOMID). Anakinra differs from native human IL-1Ra in that it has the addition of a single methionine residue at its amino terminus. Anakinra consists of 153 amino acids and is a large molecule with a molecular weight of 17.3 kilodaltons. Anakinra is produced by recombinant DNA technology using an Escherichia coli bacterial expression system.

2.2 Summary of the biological effects of anakinra

IL-1 is a pleiotropic cytokine produced primarily by monocytes and macrophages in response to inflammatory stimuli, which mediates various inflammatory and immunological responses[20]. The biological effects of IL-1 are in part regulated by IL1-Ra. The natural IL-1Ra is produced primarily by monocytes and macrophages in response to a wide range of stimuli (e.g., lipopolysaccharide[21], bacterial endotoxin[22], IL-1 alpha, IL-3, IL-4 and GM-CSF[23]). IL-1Ra competitively binds to both type I and type II IL-1 receptors, at least partially blocking cellular responses mediated by IL-1-alpha and IL-1-beta. Compared to natural IL-1Ra, anakinra retains a comparable ability to inhibit IL-1 binding in vitro.[24,25] In vitro studies showed that a 50% inhibition of IL-1-induced biological responses required amounts of anakinra up to 100-fold in excess of the amounts of IL-l alpha or IL-1 beta present[26]. In a primate model, a 1,000 fold molar excess of anakinra could successfully inhibit the hemodynamic effects of IL-1-alpha administration[27].

2.3 Rationale for the use of anakinra to prevent CRS And ICANS after CD19 CAR T-cell therapy

2.3.1 Preclinical data

Using immunodeficient beige mice receiving intraperitoneal injection of Raji cells followed by human CD19 CAR T cells, Giavridis et al[28] induced CRS, but only in mice who developed high tumor burden. Importantly, the authors failed to induce CRS under the same conditions using NSG mice, which are known to harbor development and maturation defects of monocytes and macrophages. In the beige mice, human CD19 CAR T cells activated peritoneal macrophages, which were the main source of IL-6. Treatment with a blocking antibody against the murine IL-6R in this model prevented CRS-related mortality. Furthermore, peritoneal macrophages produced high levels of inducible nitric oxide synthase (iNOS), and CRS severity could be diminished using iNOS inhibitors. Since iNOS production was both IL-6 and IL-1-dependent, the authors administered anakinra intraperitoneally to antagonize the IL-1R, which successfully prevented CRS while preserving anti-tumor efficacy. Despite the limitations of a non-humanized mouse model (e.g., peritoneal disease, murine myeloid lineage, anakinra administered intraperitoneally instead of subcutaneously in humans), this data suggest anakinra may be efficacious at preventing or treating CRS.

Norelli et al[29] described a humanized mouse model more closely reflecting human biology, by providing bystander human hematopoiesis and generating xenotolerant CAR T cells. To achieve this, the authors transplanted human cord blood hematopoietic stem and progenitor cells into sublethally irradiated triple transgenic NSG (SGM3) mice expressing human stem cell factor, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-3. CAR T cells were then engineered from human T cells that matured in the newborn humanized SGM3 mice (nHuSGM3). Intravenous infusion of CD19 or CD44v6-targeted nHuSGM3 CAR T cells did not cause CRS in non-humanized SGM3 mice. In contrast, when infused into humanized mice CAR T cells caused a significant systemic inflammatory syndrome resembling CRS, characterized by severe weight loss, increased systemic human IL-6, TNF-α, IL-10 levels and high fever. In this model, CRS occurred in the absence of tumor, due to the presence of normal human CD19+ B cells and CD44v6+ myeloid cells, but was worsened in the presence of tumor. Single cell RNA sequencing revealed that human monocytes were the predominant source of IL-1 and IL-6 during CRS. They observed distinct cytokine kinetics, IL-1 production preceding IL-6 production by approximately 24 hours. Although both IL-1R and IL-6R blockage prevented CRS without impacting CAR T-cell expansion or antitumor effects, only anakinra prevented the development of fatal ICANS. [28,29]. These findings strongly suggest the early administration of anakinra could abrogate CRS and ICANS after CD19 CAR T-cell therapy.

2.3.2 Clinical data

Anakinra treatment was shown to be effective in treating CRS associated with hemophagocytic lymphohistiocytosis (HLH) features in children and young adults with B-ALL receiving CD22-targeted CAR T cells[30]. After incorporation of CD8+ and CD4+ T cell selection (TCS) in the starting leukapheresis material at the second dose level (DL2), more participants developed HLH-like manifestations (DL2, 3 of 18; DL2-TCS, 5 of 7; P = .017), despite a similar incidence and grade of CRS, with a higher frequency of participants at DL2-TCS developing coagulopathy. HLH-like toxicities occurred only in participants who experienced CRS; 19 (38%) of 50 participants with CRS developed HLH-like manifestations. The average time to onset of HLH-like features was 14 days (range, 7-26 days) post-CAR, and CRS was generally resolved or resolving before the onset of HLH-like manifestations. The incidence of HLH-like toxicities was higher in those who underwent TCS (16 [55.2%] of 29 versus 3 [14.3%] of 21; P = .0039). Peak ferritin was substantially higher in those at DL2 versus DL2-TCS and among all who received a product with TCS, with a median ferritin of 163,200 mg/L (range, 5,769-565,510 mg/L) versus 14,349 mg/L (range, 106-590,100 mg/L; P = .0007). Peak IL-1β serum concentrations were significantly higher in

patients who received CAR T cell products with TCS compared to patients who received a bulk product (median, 1.1pg/mL versus 0.7 pg/mL; IQR, 0.8-7 pg/mL versus 0.5-1.1 pg/mL, respectively; p=0.030). HLH-directed treatment was initiated in 14 of 58 participants (24%) because of worsening laboratory parameters or clinical symptoms (e.g., pulmonary edema; renal dysfunction; worsening coagulopathy; steadily increasing inflammatory markers concerning for a worsening trajectory or symptomatic global inflammation, such as noninfectious cholecystitis). Systematic use of anakinra at starting doses of 5-8 mg/kg/day subcutaneously was incorporated to treat or prevent worsening of HLH-like manifestations in participants with clinically relevant findings on the basis of data in treatment of secondary HLH. Treatment was initiated with anakinra alone (n = 3), corticosteroids plus anakinra (n = 5), or corticosteroids alone (n = 6). All treated participants had resolution of HLH-like toxicities without any apparent negative impact on response or CAR T-cell expansion. In one participant, HLH-like manifestations developed at day 28 after bone marrow restaging revealed a minimal residual disease–positive CR. Within 1 month of anakinra monotherapy, all laboratory abnormalities normalized, and subsequent restaging demonstrated ongoing CAR activity with eradication of MRD. The efficacy of anakinra on ICANS symptoms was not specifically reported.

A retrospective case series (n=8) by Strati et al suggested anakinra may be efficacious in treating severe or refractory CRS and ICANS in LBCL patients receiving axi-cel[31]. The indication for the use of anakinra was high-grade ICANS in 6 patients and HLH in 2 patients. At the time of initiation of anakinra, the median cumulative dexamethasone equivalent dose was 273 mg (range, 0-1344), and the median cumulative tocilizumab dose was 1080 mg (range, 0-1480; with a median of 2 doses of 8 mg/kg each). Anakinra was started at a median of 12 days (range, 6-41) after axi-cel infusion, with a median daily dose of 100 mg (range, 50200 mg) subcutaneously, for a median of 7 days (range, 1-7) and a median cumulative dose of 700 mg (range, 200-700 mg). Although none of the patients continued to receive tocilizumab after initiation of anakinra, all continued to receive corticosteroids, for a cumulative dexamethasone equivalent dose of 722 mg (range, 10-1545 mg). Overall, 4 patients (50%) had a clinical response after initiation of anakinra, and 4 (50%) were refractory. In all 4 responders, the indication for use of anakinra was high-grade ICANS, and all received 100 mg SC daily for 7 days. Patient 1 started anakinra on day 6 for grade 4 ICANS (after a cumulative dexamethasone dose of 336 mg and a cumulative tocilizumab dose of 1480 mg), with durable conversion to low-grade ICANS on day 10 and a decrease to grade 0 on day 22 (a cumulative dexamethasone dose of 1081 mg was provided during treatment with anakinra). The patient achieved complete response (CR) on day 30 and was in remission 9 months after axi-cel infusion. Patient 2 started anakinra on day 41 for grade 3 ICANS (after a cumulative dexamethasone dose of 1344 mg and a cumulative tocilizumab dose of 1180 mg), with durable conversion to low-grade ICANS on day 46 (a cumulative dexamethasone dose of 333 mg was provided during treatment with anakinra). The patient died of severe pneumonia on day 80, while in CR and with persistent grade 1 ICANS (mild cognitive impairment). Patient 3 started anakinra on day 31 for grade 3 ICANS (with no previous exposure to corticosteroids and/or tocilizumab), with a decrease to grade 0 on day 35 (a single dose of dexamethasone 10 mg was provided during treatment with anakinra), but died of HLH on day 71, while in CR. Patient 4 started anakinra on day 7 for grade 4 ICANS (after a cumulative dexamethasone dose of 140 mg and a cumulative tocilizumab dose of 1400 mg), with temporary conversion to lowgrade ICANS on day 13 (a cumulative dexamethasone dose of 1336 mg was provided during treatment with anakinra). After the patient completed 7 days of anakinra, high-grade (grade ≥3) ICANS recurred on day 16, and he died of progressive LBCL on day 18.

In the pivotal clinical trial ZUMA-1, investigating the use of axi-cel in patients with relapsed or refractory LBCL, higher IL-1R antagonist peak serum concentrations were associated with CRS and ICANS severity[32].

An interim analysis of the first 15 patients treated on this protocol with subcutaneous (SC) anakinra, all patients received all doses of anakinra as planned, without dose adjustments or interruptions, and there were no adverse events (AEs) attributed to anakinra. The rates of any grade CRS and ICANS were 53% (≥ grade 3: n = 1) and 20% (all ≥ grade 3), respectively, which are lower than those in patients treated at our institution who received liso-cel without prophylactic anakinra (CRS: 68%; ICANS: 27%). We also observed faster resolution of inflammatory biomarkers, such as temperature, CRP, and IL-6. Day+28 complete and overall response rates were comparable in the two cohorts (complete response rate: 33% in anakinra cohort vs. 45% in no-anakinra cohort; overall response rate: 75% in anakinra cohort vs. 85% in no-anakinra cohort).

2.4 Rationale for anakinra dosing, administration route, treatment duration, up-titration

Dosing

We hypothesize the low RA flat dose of 100 mg/day may not be sufficient to mitigate ICANS after CD19 CAR T-cell therapy. Anakinra is a large 17kD-molecule with low permeation into the CSF; high doses of anakinra are needed to achieve neuroprotective concentrations in rhesus monkey models of NOMID[33], and in humans developing subarachnoid hemorrhage[34]. NOMID, which is characterized by chronic aseptic meningitis, typically respond to higher doses of anakinra (1-8 mg/kg/day). As described above, higher anakinra doses (5-8 mg/kg/day) have been used safely and successfully to treat CAR T-cell related CRS with HLH features[30]. Very high doses of anakinra were also shown to be safe in patients with sepsis[35] (1-2 mg/kg/h intravenously (IV), acute GVHD[36-38] (up to 3200 mg/day IV), stroke[39] (2 mg/kg/h IV), and subarachnoid hemorrhage[34,40] (100-500 mg bolus, then 2-10 mg/kg/h IV). In healthy human subjects anakinra doses up to 10 mg/kg IV are not associated with clinical symptoms, and the authors did not report impairment of in vitro T cell proliferation[41].

Pharmacokinetic studies in healthy adults across a spectrum of body weights have demonstrated that the maximum plasma concentration of anakinra (C_{max}) is up to 30-fold higher with IV administration compared to SC administration (~20,000-30,000 ng/mL vs. ~600-1,000 ng/mL). In addition, the area under the concentration-time curve (AUC) was also higher in patients receiving IV anakinra (~10,000-15,000 ng-hour/mL vs. ~8,000-13,000 ng-hour/mL). No new safety concerns were observed[42].
Therefore, we hypothesize that the IV route will improve the efficacy of anakinra to prevent CRS and ICANS. Anakinra 200 mg will be diluted in 200 mL of sodium chloride 0.9% and administered over approximately 10-30 minutes once daily (prophylactic dose), which can be practically administered in the outpatient setting. Given the encouraging safety data and response rates in patients who received SC anakinra, we do not anticipate significant AEs or impact on CAR T-cell efficacy associated with IV administration.

Administration schedule

To assess our primary objective – to decrease the incidence of CRS of any grade after treatment with liso-cel – anakinra will be administered IV, first at the prophylactic dose of 200 mg/day for 14 days (through day +13 after liso-cel infusion). The first dose of anakinra will be administered approximately 2-4 hours prior to CAR T-cell infusion.

Treatment duration
Since ICANS after CD19 CAR T-cell therapy is in most cases delayed (median time to onset, 5-9 days) and prolonged (median duration, 7-14 days), anakinra will be administered for 14 days after liso-cel infusion.

Up-titration of anakinra during CRS and ICANS

Given the high variance in the degree of systemic inflammation observed across patients during CRS and ICANS, we hypothesize a two-step, clinically driven, up-titration of the anakinra dose will decrease CRS/ICANS severity and accelerate symptom resolution. Up-titration of anakinra has been previously reported to treat CRS or ICANS[43], and secondary HLH[44]. Details regarding up-titration of the anakinra dose are provided in Section 6.1.

2.5 Rationale for the use of a single CD19 CAR T-cell product

Pivotal clinical trial data suggest significant differences in the incidence and severity of CRS and ICANS across FDA-approved CAR T-cell products. Since this is a pilot study with limited sample size, we will restrict the inclusion criteria to patients treated with liso-cel to analyze a population with a more homogeneous risk of CRS/ICANS.

3.0 STUDY OBJECTIVES AND ENDPOINTS

3.1 Primary Objective

Decrease the incidence of CRS by day 28 after liso-cel treatment for B-NHL.

3.2 Secondary Objectives

1. Decrease the severity of CRS by day 28 after liso-cel treatment .
2. Decrease the incidence and severity of neurotoxicity by day 28 after liso-cel treatment.
3. Decrease the rate and duration of hospitalization
4. Decrease corticosteroid usage after liso-cel treatment
5. Evaluate safety of anakinra use after CAR-T cell infusion.
6. Evaluate the effect of anakinra on liso-cel efficacy for treatment of B-NHL, as evaluated at approximately day 28 and day 90 after treatment.

3.3 Exploratory Objectives

1. To assess the pharmacokinetics of anakinra in B-NHL patients undergoing treatment with liso-cel
2. To evaluate the effects of anakinra on the immune system (e.g., CAR T cells in vivo kinetics, other immune cells, serum cytokine concentrations)

3.4 Primary Endpoint

Absence of any grade CRS

3.5 Secondary Endpoints

- Peak CRS grade according to the 2019 American Society for Transplantation and Cellular Therapy (ASTCT) consensus criteria[45] within 28 days after liso-cel infusion. CRS will also be graded according to the 2014 NIH criteria[46] to allow comparisons to historical controls.
- Peak ICANS grade according to the 2019 ASTCT consensus criteria[45] within 28 days after liso-cel infusion. ICANS will also be graded according to the NCI CTCAE Version 5.0 to allow comparisons to historical controls.
- Rate and duration of hospitalization after liso-cel treatment.
- Cumulative corticosteroids dose within 28 days after liso-cel infusion.
- Adverse events (AEs) according to the NCI CTCAE Version 5.0 within the first 28 days after liso-cel administration.
- Disease response to CAR T-cell therapy approximately 28 days and 90 days after CAR T-cell infusion according to the Lugano criteria[47].

3.6 Projected Target Accrual

Table 1: Targeted / Planned Enrollment

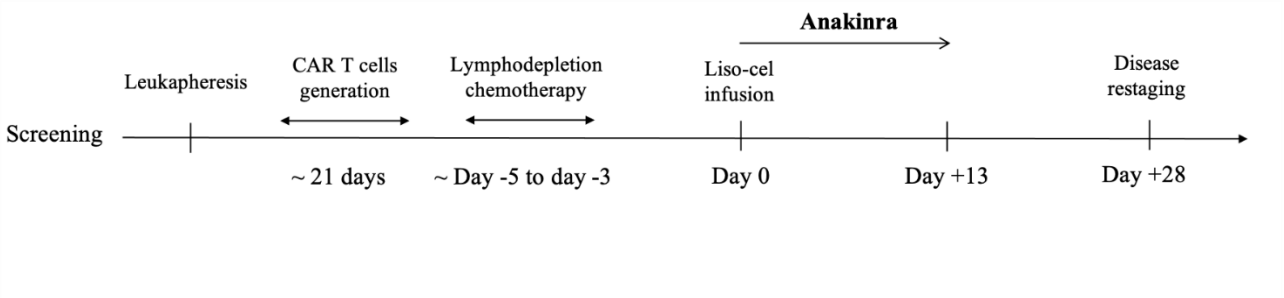
Ethnic Category	**Patient Numbers**		
	Females	**Males**	**Total**
Hispanic or Latino	0	0	0
Not Hispanic or Latino	10	15	25
Ethnic Category Total of All Subjects	10	15	25
Racial Category			
American Indian / Alaska Native	0	0	0
Asian	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	0	0	0
White	10	15	25
More than One Race	0	0	0
Racial Categories: Total of All Subjects	10	15	25

4.0 STUDY DESIGN AND INVESTIGATIONAL PLAN

4.1 Overall Study Design

Phase 2 single-center pilot study to evaluate the efficacy and safety of anakinra to prevent liso-cel-related CRS and neurotoxicity

Figure 1. Study Schema



4.2 Protocol Enrollment

Enrollment is expected to take approximately 2 years. Disease restaging should be performed per standard of care approximately 28 and 90 days after liso-cel infusion. The study team may contact the local oncologist office by a letter/phone call to request the results of the day 90 restaging.

5.0 STUDY POPULATION

The target study population consists of adult patients with B-NHL receiving treatment with liso-cel. Patients must meet all of the inclusion criteria and have none of the exclusion criteria to be enrolled in this study.

5.1 Inclusion Criteria

Results of tests and/or procedures conducted as per standard of care purposes may be used for research purposes if conducted within the protocol-defined window prior to screening/leukapheresis and/or T-Cell Therapy.

1) Subjects must be 18 years of age or older.
2) Karnofsky performance status of ≥ 60%.
3) Patients with B-NHL and eligible for treatment with liso-cel. Patients treated with non-conforming (out-of-specification) liso-cel may remain on study.
4) Negative serum pregnancy test within 2 weeks of enrollment for women of childbearing potential, defined as those who have not been surgically sterilized or who have not been free of menses for at least 1 year.
5) Fertile male and female subjects must be willing to use an effective contraceptive method before, during, and for at least 4 months after the last dose of anakinra.
6) Ability to understand and provide informed consent.

5.2 Exclusion criteria

1) Subjects requiring ongoing daily corticosteroid therapy at a dose of >15 mg of prednisone per day (or equivalent). Pulsed corticosteroid use for disease control is acceptable.
2) Active autoimmune disease requiring immunosuppressive therapy is excluded unless discussed with the PI.
3) Known hypersensitivity to E coli-derived proteins, anakinra, or to any component of the product.
4) Major organ dysfunction defined as:

a. Serum creatinine > 2.5 mg/dL
b. Significant hepatic dysfunction (Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) > 5x upper limit of normal; bilirubin > 3.0 mg/dL) unless due to malignancy or Gilbert's syndrome in the opinion of the PI or designee.
c. Subjects with clinically significant pulmonary dysfunction, as determined by medical history and physical exam should undergo pulmonary function testing. Those with an FEV1 of < 50 % of predicted or DLCO (corrected) < 40% will be excluded.
d. Significant cardiovascular abnormalities as defined by any one of the following: NYHA class III or IV congestive heart failure, clinically significant hypotension, uncontrolled symptomatic coronary artery disease, or a documented ejection fraction of <35%.

5) Uncontrolled serious and active infection.

5.3 Reproductive Potential and Contraception Requirements

Pregnancy test for females of reproductive potential must be negative within 2 weeks before enrollment. Any female patient who does not meet at least one of the following criteria will be considered to have reproductive potential:

- Post-menopausal for at least 12 consecutive months (i.e., no menses), or
- Undergone a sterilization procedure (hysterectomy, salpingectomy, or bilateral oophorectomy; tubal ligation is not considered a sterilization procedure)

Female subjects with reproductive potential who are not sexually abstinent and male subjects who are sexually active with females of reproductive potential must agree to use a suitable method of contraception for the duration of the study and for at least 4 months after the last dose of anakinra, for example:

- Condom with spermicidal agent
- Diaphragm or cervical cap with spermicidal agent
- Intrauterine device
- Hormonal contraceptives in combination with either a condom, diaphragm, or cervical Cap

6.0 TREATMENT PLAN

6.1 Anakinra Treatment

Prophylactic dose:

Anakinra 200 mg will be diluted in 200 mL of sodium chloride 0.9% and administered IV over approximately 10-30 minutes once daily (approximately every 24 hours,) for 14 days, with the first dose administered 2-4 hours before liso-cel infusion (day 0 through day 13). Scheduling variations in the timing of anakinra administration are acceptable, e.g., in case of clinical events (e.g., unplanned hospitalization) or if changes to the patient's schedule occur. The anakinra dose may be modified or held at the discretion of the PI or designee (e.g., in case of severe and/or uncontrolled infection). Should patients receive the first dose of anakinra but do not receive liso-cel for any reason, they may be re-treated with anakinra if liso-cel is given at a later point.

Anakinra dose modification: in patients with severe renal insufficiency or end-stage renal disease, defined as CrCl less than 30 mL/min, anakinra will be administered every other day instead of every day. Local injection-site reactions should be managed per institutional standards.

Up-titration in case of CRS or ICANS:

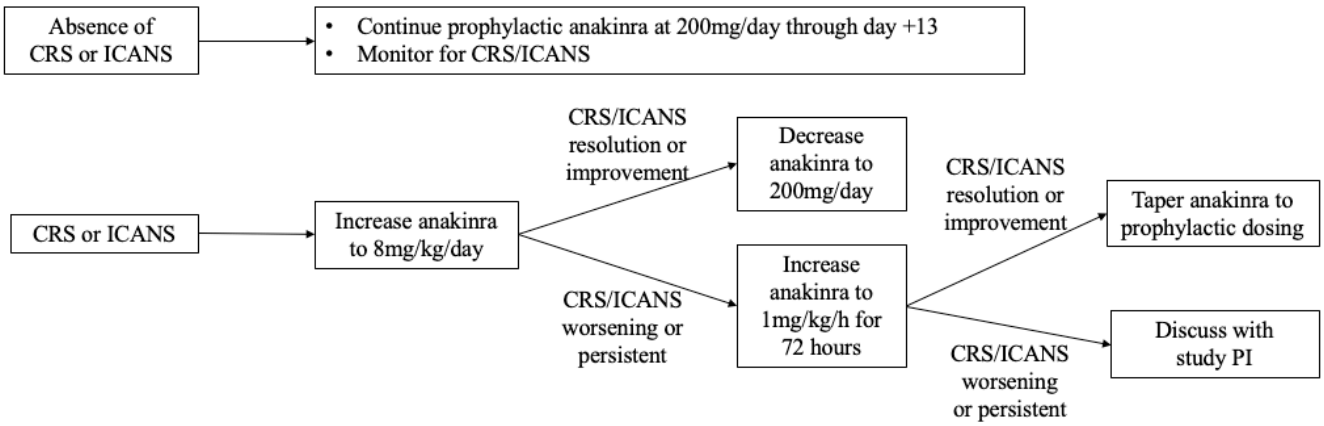
Step 1 (8 mg/kg/day): In patients who develop CRS or ICANS during anakinra prophylaxis at 200 mg/day, anakinra will be increased to 8 mg/kg/day. The step 1 anakinra dose will be calculated using the participant's actual or ideal body weight as per PI discretion. The total daily dose will be divided over three doses in 24 hours, each dose will be rounded to the nearest 100 mg, and diluted in NaCl 0.9% at a volume sufficient to achieve a goal concentration of 1 mg/ml, and given IV over approximately 10-30 minutes. Once CRS and ICANS symptoms are improved or resolved, as evaluated by the PI or designee, anakinra will be decreased to the prophylactic dosing (200 mg/day).

Anakinra dose modification: in patients with severe renal insufficiency or end-stage renal disease, defined as CrCl less than 30 mL/min, the anakinra dose will be reduced to 50% of the target dose (4 mg/kg/day).

Step 2 (1 mg/kg/h): In the absence of improvement or worsening of CRS or ICANS symptoms despite anakinra treatment at 8 mg/kg/day, as evaluated by the PI or designee, anakinra will be increased once to 1 mg/kg/h administered as a continuous IV infusion for 72 hours. The step 2 anakinra dose will be calculated using the participant's actual or ideal body weight as per PI discretion, rounded to the neared 100 mg, and diluted in NaCl 0.9% at a volume sufficient to achieve a goal concentration of 1 mg/ml. After 72 hours of anakinra at 1 mg/kg/h, anakinra will be decreased to 8 mg/kg/day. Once CRS and ICANS symptoms are improved or resolved on anakinra at 8 mg/kg/day, as evaluated by the PI or designee, anakinra will be further decreased to the prophylactic dosing.

Anakinra dose modification: in patients with severe renal insufficiency or end-stage renal disease, defined as CrCl less than 30 mL/min, the anakinra dose will be reduced to 50% of the target dose (0.5 mg/kg/h).

Anakinra up-titration algorithm:



6.2 Anakinra Drug Product

Anakinra is supplied in single use 1 mL prefilled glass syringes with 27 gauge needles as a sterile, clear, colorless-to-white, preservative- free solution for daily subcutaneous (SC) administration. Each 1 mL prefilled glass syringe contains: 0.67 mL (100 mg) of anakinra in a solution (pH 6.5) containing sodium citrate (1.29 mg), sodium chloride (5.48 mg), disodium EDTA (0.12 mg), and polysorbate 80 (0.70 mg) in Water for Injection, USP. Anakinra will be supplied by Sobi and dispensed through Investigational Drug Services.

6.3 Liso-cel treatment

Liso-cel will be infused per institutional practice and according to the Breyanzi Risk Evaluation and Mitigation Strategies (REMS)[48].

7.0 POTENTIAL RISKS OF ANAKINRA TREATMENT

Injection-site Reactions
The most common and consistently reported treatment-related adverse event associated with anakinra is injection-site reaction (ISR). In clinical trials using anakinra for treatment of Rheumatoid Arthritis 71% of patients developed an ISR, which was typically reported within the first 4 weeks of therapy. The majority of ISRs were reported as mild (72.6% mild, 24.1% moderate and 3.2% severe). The ISRs typically lasted for 14 to 28 days and were characterized by 1 or more of the following: erythema, ecchymosis, inflammation, and pain.

Hypersensitivity Reactions
Hypersensitivity reactions including anaphylactic reactions, angioedema, urticaria, rash, and pruritus have been reported with anakinra.

If a severe hypersensitivity reaction occurs, administration of anakinra should be discontinued and appropriate therapy initiated according to institutional or clinical standard.

Infections
In clinical trials using anakinra for treatment of Rheumatoid Arthritis, the incidence of infection was 39% in the anakinra-treated patients and 37% in placebo-treated patients during the first 6 months of blinded treatment. The incidence of serious infections was 2% in anakinra-treated patients and 1% in patients receiving placebo over 6 months. The incidence of serious infection over 1 year was 3% in anakinra-treated patients and 2% in patients receiving placebo. These infections consisted primarily of bacterial events such as cellulitis, pneumonia, and bone and joint infections. Majority of patients (73%) continued on study drug after the infection resolved. No serious opportunistic infections were reported. Patients with asthma appeared to be at higher risk of developing serious infections when treated with anakinra (8 of 177 patients, 4.5%) compared to placebo (0 of 50 patients, 0%). In open-label extension studies, the overall rate of serious infections was stable over time and comparable to that observed in controlled trials. In clinical studies and post marketing experience, cases of opportunistic infections have been observed and included fungal, mycobacterial and bacterial pathogens. Infections have been noted in all organ systems

and have been reported in patients receiving anakinra alone or in combination with immunosuppressive agents.

Antimicrobial prophylaxis and infection surveillance and treatment will be according to institutional standard for patient receiving CD19-targeted CAR-T cell therapy

Malignancies
Among 5300 Rheumatoid Arthritis patients treated with anakinra in clinical trials for a mean of 15 months (approximately 6400 patient years of treatment), 8 lymphomas were observed for a rate of 0.12 cases/100 patient years. This is 3.6 fold higher than the rate of lymphomas expected in the general population, based on the National Cancer Institute's Surveillance, Epidemiology and End Results (SEER) database (SEER Incidence Crude Rates, 11 Registries, 1992-1999) An increased rate of lymphoma, up to several fold, has been reported in the Rheumatoid Arthritis population, and may be further increased in patients with more severe disease activity. Thirty-seven malignancies other than lymphoma were observed. Of these, the most common were breast, respiratory system, and digestive system. There were 3 melanomas observed in one Rheumatoid Arthritis study and its long-term open-label extension, greater than the 1 expected case. The significance of this finding is not known. While patients with Rheumatoid Arthritis, particularly those with highly active disease, may be at a higher risk (up to several fold) for the development of lymphoma, the role of IL-1 blockers in the development of malignancy is not known.

Hematologic Events
In placebo-controlled studies with anakinra, 8% of patients receiving anakinra had decreases in total white blood counts of at least one WHO toxicity grade, compared with 2% of placebo patients. Nine anakinra-treated patients (0.4%) developed neutropenia (ANC < 1 x 109 /L). 9% of patients receiving anakinra had increases in eosinophil differential percentage of at least one WHO toxicity grade, compared with 3 % of placebo patients. 2% of patients receiving anakinra had decreases in platelet count, all WHO toxicity grade one, compared to 0% of placebo patients.

Immunogenicity
As with all therapeutic proteins, there is potential for immunogenicity. In Studies using anakinra for treatment of Rheumatoid Arthritis, from which data is available for up to 36 months, 49% of patients tested positive for anti-anakinra binding antibodies at one or more time points using a biosensor assay. Of the 1615 patients with available data at Week 12 or later, 30 (2%) tested positive for neutralizing antibodies in a cell-based bioassay. Of the 13 patients with available follow-up data, 5 patients remained positive for neutralizing antibodies at the end of the studies. No correlation between antibody development and adverse events was observed. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assays.

Hyperlipidemia
Cholesterol elevations were observed in some patients treated with anakinra.

7.1 Management of Other Toxicities

CRS: Patients who develop CRS will be treated according to institutional standard practice..

ICANS: Patients who develop ICANS will be treated according to institutional standard practice.

If a new onset CTCAE v.5 Grade ≥ 3 toxicity is observed, the patient will receive investigation and medical treatment appropriate for the physiological abnormalities.

8.0 INFORMED CONSENT OF SUBJECT AND DONOR

Subjects will be seen at the FHCC/UWMC for consideration of treatment options for their disease. The protocol will be discussed thoroughly with the patient and other family members if appropriate, and all known and potential risks to the patient will be described. The procedure and alternative forms of therapy will be presented as objectively as possible, and the risks and hazards of the procedure explained to the patient. Consent from the patient will be obtained using forms approved by the Cancer Consortium IRB. A summary of the clinic visit detailing what was covered will be dictated for the medical record.

9.0 SUBJECT REGISTRATION

Eligible subjects will be identified and registered into the system by the Clinical Coordinator's Office (CCO; Intake Office) and assigned a Unique Patient Number (UPN). The CCO will register the patient for the protocol through the Data Management Office.

Enrollment to the study will occur after data are reviewed for all inclusion and exclusion criteria by the Immunotherapy attending physician and the patient signs the study consent.

A study number will be allocated to each patient and a log of enrolled subjects will be maintained.

10.0 CLINICAL AND LABORATORY EVALUATIONS

10.1 Screening & prelymphodepletion evaluations

The following evaluations should be conducted within 6 weeks prior to lymphodepletion:

1) Medical history and physical examination including height and weight
2) Karnofsky performance status
3) Laboratory tests, including:
   a. CBC, differential, platelet count
   b. Renal/Hepatic Function Panel
   c. Serum ferritin
   d. Serum CRP
   e. Uric acid
   f. Quantitative serum IgG level
   g. Prothrombin time (PT), partial thromboplastin time (PTT)
   h. Triglycerides (non-fasting levels are acceptable)
   i. Serum pregnancy test for females of childbearing potential within 2 weeks of planned enrollment
4) Baseline pulse oximetry and documentation of O_2 saturation on room air

The following evaluations should be conducted within 3 months prior to lymphodepletion:

5) Baseline chest x-ray
6) Baseline 12-lead EKG

7) Echocardiogram or MUGA scan
8) Pulmonary function testing, if clinically indicated
9) CT/PET or CT
10) Bone marrow aspirate and biopsy, if clinically indicated
11) Lumbar puncture for CSF analysis, if clinically indicated

10.2 Evaluations on the day of liso-cel infusion (day 0)

The below tests will be done as part of the clinical evaluation prior to liso-cel infusion::

1. Interval history and physical examination
2. ICE score
3. Recording of CRS and ICANS-related interventions
4. Recording of AEs
5. Blood draw for laboratory studies:

    a. CBC, differential, and platelets
    b. Renal/Hepatic Function Panel
    c. Uric acid
    d. Serum ferritin
    e. Serum CRP
    f. Serum IL-6 level
    g. DIC panel without platelets (PT, PTT, fibrinogen, D-dimer)
    h. Triglycerides (non-fasting levels are acceptable)

10.3 Evaluations following liso-cel infusion

The proposed days of all assessments and laboratory evaluations are approximate and may vary due to scheduling, clinical factors, research priorities or other considerations. All timepoints may not be collected. Laboratory research evaluations may vary according to clinical and research priorities.
The following evaluations will be performed as part of the clinical evaluation after liso-cel infusion:

1) Interval history and physical examination approximately on day +1, +3, +7, +10, +14, +21, +28
2) ICE score approximately on day +1, +3, +7, +10, +14, +21, +28
3) Continuous recording of CRS and ICANS-related interventions through day +28
4) Continuous recording of AEs through day +28 as described in section XX
5) The following laboratory studies approximately on day +1, +3, +7, +10, +14, +21, +28:

    a. CBC, differential, and platelet count
    b. Renal/Hepatic Function Panel
    c. C-reactive protein (CRP)
    d. Serum ferritin
    e. Serum IL-6 level
    f. DIC panel without platelets (PT, PTT, fibrinogen, D-dimer)
    g. Triglycerides (non-fasting levels are acceptable)

6) If subjects become febrile or develop symptoms of cytokine release or tumor lysis between the indicated time points, serum ferritin, CRP, DIC panel, and tumor lysis markers at additional times, may be measured as clinically indicated.

10.4 Evaluations before and after each anakinra administration

Vital signs including pulse oximetry before and 30 minutes after each anakinra administration on days 0 through day +13. For patients receiving step-up dosing, vital signs will be obtained every 4 hours per standard-of-care.

10.5 Research Blood samples

Pharmacokinetic samples

- Approximately 4 hours after the end of the first anakinra administration, up to 10mL of blood may be obtained
- Approximately 24 hours (+/- 1 hour) after the end of the first anakinra administration and immediately prior to the second anakinra administration, up to 10mL of blood may be obtained

Details regarding the tube type, handling, receiving laboratory, are detailed in a separate Research Sample Manual.

Because the clinical treatment and follow-up schedule may differ between patients, variation in the proposed blood sampling schedule is acceptable. It is also acceptable to collect fewer samples and/or less than the maximum sample number or volume depending on clinical, logistical and/or research priorities. If the patient's schedule is modified, samples may be recollected.

Tissue/fluid samples

If sampling of tissues or fluids (e.g., cerebrospinal fluid, pleural fluid, ascites, skin) is performed for clinical indications (e.g., in the context of CRS or ICANS), an additional sample may be obtained during the same procedure and sent to the FHCC for research studies. The planned procedure should be discussed with the PI.

Additional research samples

In consenting patients, other research samples may be obtained using the sample acquisition partner protocol FHCC 8682 "Collection of Samples and Data from Patients Undergoing Treatment with Commercial Chimeric Antigen Receptor Engineered T Cells".

10.6 Assessment of Disease Response to CAR-T Cell Therapy

Objective responses to the therapeutic regimen will be assessed approximately 28 days and 90 days after liso-cel infusion using physical examination, imaging studies, and if necessary, bone marrow biopsies, as clinically indicated. If CT or PET/CT data are available, response will be defined per the Lugano criteria[47]. The study team may contact the local oncologist office by a letter/phone call to request the results of the day 90 restaging.

10.7 Long-Term Follow-up

Subjects will be followed per institutional standard of care.

11.0 ADVERSE EVENT REPORTING

11.1 Adverse Event Definitions

- **Adverse Event**
  An Adverse Event (AE) is any untoward medical occurrence in a clinical investigation subject administered a medicinal product; the event does not necessarily have a causal relationship with study drug administration or usage. An adverse event can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

- **Serious Adverse Event**
  A serious adverse event (SAE) is defined as an untoward medical occurrence that results in any of the following outcomes:
  1. Death.
  2. Life-threatening situation (i.e., with an immediate risk of death from the event as it occurred but not including an event that, had it occurred in a more serious form, might have caused death).
  3. In-patient hospitalization or prolongation of existing hospitalization. Inpatient hospitalization comprises formal admission to a hospital for medical reasons, for any length of time, whether or not hospitalization extends overnight. However, hospital admissions for administration of the study drug, procedures required by the study protocol, or tumor-related diagnostic procedures are not considered serious.
  4. Persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions.
  5. Congenital anomaly/birth defect.
  6. An important medical event that requires intervention to prevent one of the above outcomes.

- **Unexpected Adverse Event**
  An unexpected adverse event is defined as an event that has a nature or severity, or frequency that axis not consistent with the investigator brochure, protocol, or consent form.

11.2 Monitoring and Recording Adverse Events

Grade ≥3 AEs will be assessed by the investigator or qualified designee and recorded in the CRFs. In addition, grade 1-2 AEs contributing to the diagnosis and grading of CRS (including for example, fever, hypotension, hypoxia) and/or neurologic symptoms contributing to the diagnosis and grading of ICANS (including for example, delirium, seizure, encephalopathy) will be collected. The investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the adverse event and/or serious adverse event and not described as the individual signs or symptoms. The following information should be recorded:

- Description of the adverse event using concise medical terminology
- Description as to whether or not the adverse event is serious
- The start date (date of adverse event onset)
- The stop date (date of adverse event resolution or return to baseline)

- The severity (grade) of the adverse event
- A description of the potential relatedness of the adverse event to study drug or a study procedure
- Expectedness of the adverse event based on prior observed and documented adverse events
- The outcome of the adverse event

11.3 Grading of the Severity of an Adverse Event

AEs will be graded in severity according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0. If a CTCAE criterion does not exist, the investigator should use the grade or adjectives: Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe), Grade 4 (life-threatening), or Grade 5 (fatal) to describe the maximum intensity of the adverse event. CRS will be graded according to the 2019 ASTCT consensus criteria[45] (Appendix A) and the 2014 NIH Lee criteria[46] (Appendix B), the latter to allow for comparisons with historical controls. ICANS will be graded according to the 2019 ASTCT criteria (Appendix C) and the CTCAE 5.0, the latter to allow for comparisons with historical controls. In addition, therapeutic interventions related to CRS and ICANS management will be recorded.

11.4 Attribution of Adverse Event

Association or relatedness to the study agent will be assessed by the investigator as follows:

Definite (must have all 4)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions • Follows a known pattern of response to intervention • Disappears or decreases with reduction in dose or cessation of intervention and recurs with re-exposure
Probable (must have 3)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state or have been due to environmental or other interventions • Follows a known pattern of response to intervention • Disappears or decreases with reduction in dose or cessation of intervention
Possible (must have 2)	• Has a reasonable temporal relationship to the intervention • Could not have readily been produced by the subject's clinical state • Could not readily have been due to environmental or other interventions • Follows a known pattern of response to intervention
Unlikely (must have 2)	1. Does not have a temporal relationship to the intervention 2. Could readily have been produced by the subject's clinical state 3. Could have been due to environmental or other interventions 4. Does not follow a known pattern of response to intervention 5. Does not reappear or worsen with reintroduction of intervention

Unrelated	• The AE is clearly NOT related to the intervention

For AE assessment, an AE is considered related if it is assessed as definitely, probably, or possibly related; unrelated if it is assessed as unlikely related or unrelated.

11.5 Adverse Event Reporting Period

Adverse events will be monitored and recorded in study-specific case report forms (CRFs) throughout the investigative phases of the study: from the first dose of Anakinra through day 28 after the liso-cel infusion. Related AEs will be monitored until stabilization or resolution.

A subject withdrawn from the study because of an adverse event must be followed until the clinical outcome from the adverse event is determined.

The following events are *not* identified as AEs in this study:

- Disease progression or relapse.
- Medical or surgical procedures in and of themselves, including those that require hospitalization (e.g., surgery, endoscopy, biopsy procedures) are not considered AEs. However, an event or condition requiring such procedures may be an AE.

11.6 Adverse Event Reporting Requirements

The investigator or designee will report events to the Fred Hutch IRB in accordance with the policies of the IRB.

12.0 STATISTICAL CONSIDERATIONS

This is a pilot study to assess the efficacy of anakinra to prevent any grade CRS (primary endpoint) after liso-cel treatment in patients with B-NHL.

12.1 Primary endpoint:

We will assess the efficacy of anakinra in preventing the occurrence of any grade CRS using the Bayesian optimal phase 2 (BOP2) design [49] with a planned interim analysis after 15 patients are treated (n, interim sample size). Unlike other existing Bayesian designs, the BOP2 design explicitly controls the type I error rate, thereby bridging the gap between Bayesian designs and frequentist designs. N denote the maximum sample size.
Based on the TRANSCEND clinical trial data in LBCL patients, the incidence of any grade CRS liso-cel therapy is estimated to be approximately 40%.[7] Let p_{eff} denote the proportion of patients without any grade CRS (efficacy) and define the null hypothesis $H_0: p_{eff} \leq 0.6$, representing that the treatment is inefficacious. We assume a vague Beta(0.6,0.4) prior distribution for p_{eff}. Let y be defined as the absence of any grade CRS and the posterior probability will be computed as follows using 100,000 simulations:

$$Pr_{eff} = Pr(p_{eff}|y, n) = Beta(p_{eff}|y + 0.6, n - y + 0.4)$$

This primary endpoint will not be met if

$$Pr(p_{eff} > 0.6|data) < \lambda(\frac{n}{N})^{\alpha},$$

where λ=0.9 and α=0.51 are design parameters optimized to minimize the chance of incorrectly claiming that an efficacious treatment is not promising (i.e., type II error) under the alternative hypothesis $H_1: p_{eff} = 0.85$, while controlling the type I error rate (i.e., the probability of incorrectly claiming that the treatment is acceptable under H_0) at 0.1. This design yields a statistical power of 0.928 under H_1.

12.2 Secondary Endpoints

- Peak CRS grade within 28 days after liso-cel infusion
- Peak ICANS grade within 28 days after liso-cel infusion
- Rate and duration of hospitalization after liso-cel treatment
- Cumulative corticosteroids dose within 28 days after liso-cel infusion
- Grade ≥3 AEs according to the NCI CTCAE Version 5.0 within the first 28 days after liso-cel administration.
- Disease response to CAR T-cell therapy approximately 28 days and 90 days after CAR T-cell infusion according to the Lugano 2014 criteria[47]

12.3 Data Analysis

We will perform a first analysis when the number of enrolled patients reaches 15. The posterior probabilities of the absence of any grade CRS will be computed as described above. In our case, we will conclude that the primary endpoint is not reached if no CRS is observed in ≤ 9 patients in the first 15 patients treated. Since this is a pilot study, the decision to include the additional 10 patients might be considered based on both the primary and secondary endpoints.

If the total number of patients reaches the maximum sample size of 25, we will reject the null hypothesis and conclude that the primary endpoint is met if the number of patients without CRS is greater than 18 (19 or more patients with no evidence of any grade CRS); otherwise we conclude that the primary endpoint is not met.

February 2023 update: although our interim analysis did not meet the primary endpoint, anakinra prophylaxis was feasible, safe, and associated with low rates of mild CRS (8/15; 53%) and ICANS (3/15; 20%). Longitudinal analyses of the participants' temperature and CRP data suggested faster resolution of systemic inflammation compared to patient receiving liso-cel alone. Therefore, we will plan to treat 10 additional patients with the following protocol modification: change from SC to IV administration route, up-titration of the anakinra dose in case of breakthrough CRS or ICANS.

Statistical analysis will include descriptive statistics for which categorical variables will be summarized by number and percentage, and continuous variables will be summarized by median, standard deviation, and range. For binary endpoints, such as the proportion of patient without any grade CRS and the overall response rate (ORR), an exact 95% confidence interval using a binomial distribution will be provided. Time-to-event data will be analyzed using Kaplan-Meier method and the results will be summarized by the median with a 95% CI if appropriate.

12.4 Stopping Rules

To protect the safety of patients, the study incorporates the following stopping rules:

1. If the lower bound of a 1-sided 80% exact binomial confidence interval of death that are probably or definitely attributable to anakinra treatment is > 3%. Operationally, any of the following would trigger such a rule: 1 death out of the first ≤ 7 patients or 2 death out of the first ≤ 15 patients that are probably or definitely attributable to anakinra treatment. If the true probability of death is 1%, the probability of study suspension under the above rule is approximately 0.08; if the true probability is 15%, the probability of suspension is approximately 0.93 (probabilities estimated from 5,000 simulations).

2. If the upper bound of a 2-sided 95% exact binomial confidence interval of responses (ORR) is < 75% with 15 subjects at the interim analysis. Operationally, if there are 7 or fewer responses (ORR) among the first 15 subjects, the study will be stopped. Otherwise, the study will continue until 25 subjects are enrolled. If the true probability of ORR is 40%, the probability of stopping under the above rule is approximately 80%; if the true probability of ORR is 80%, the probability of stopping is approximately 0.5% (probabilities estimated from 5,000 simulations).

When a stopping rule is triggered, further enrollment will be suspended pending review by the study Steering Committee (as discussed in section 13.1).

13.0 DATA AND SAFETY MONITORING PLAN

13.1 Overall Scope of Monitoring Activities

Institutional support of trial monitoring will be in accordance with the FRED HUTCH/University of Washington Cancer Consortium Institutional Data and Safety Monitoring Plan (DSMP). Under the provisions of this plan, FRED HUTCH Clinical Research Support coordinates data and compliance monitoring conducted by consultants, contract research organizations, or FRED HUTCH employees unaffiliated with the conduct of the study. Independent monitoring visits occur at specified intervals determined by the assessed risk level of the study and the findings of previous visits per the institutional DSMP.

A Steering Committee, comprising the PI and/or designee, the IMTX medical director and study biostatistician, will act in an advisory capacity to the PI throughout the trial, as needed. If a stopping rule is triggered, further enrollment will be suspended pending review by the Steering Committee.

In addition, protocols are reviewed at least annually and as needed by the Consortium Data and Safety Monitoring Committee (DSMC), FRED HUTCH Scientific Review Committee (SRC) and the FRED HUTCH/University of Washington Cancer Consortium Institutional Review Board (IRB). The review committees evaluate accrual, adverse events, stopping rules, and adherence to the applicable data and safety monitoring plan for studies actively enrolling or treating subjects. The IRB reviews the study progress and safety information to assess continued acceptability of the risk-benefit ratio for human subjects. Approval of committees as applicable is necessary to continue the study.

The trial will comply with the standard guidelines set forth by these regulatory committees and other institutional, state and federal guidelines. The conduct of this trial will be further monitored by the IIRC Data and Safety Monitoring Board (DSMB) in accordance with an approved DSMB Charter.

13.2 Monitoring the Progress of Trial and Safety of Participants

The first level of trial oversight for this protocol will be provided by the Principal Investigator, the Research Nurse, and Research Coordinator(s), who will provide continuous oversight of the trial. These individuals will meet at least monthly to review recently acquired data, stopping rules, and adverse events. Serious adverse events will be reviewed upon occurrence to ensure prompt and accurate reporting to the Institutional Review Board (IRB). The data recorded in the research charts and protocol database will be compared with the actual data available from the medical record and/or clinical histories. Data detailed in the research case report forms (CRFs) will include the nature and severity of all toxicities. The Principal Investigator and all other investigators on the protocol have received formal training in the ethical conduct of human research.

14.0 DATA MANAGEMENT/CONFIDENTIALITY

The medical record containing information regarding treatment of the patient will be maintained as a confidential document, within the guidelines of the Fred Hutchinson Cancer Center and the University of Washington Medical Center.

The investigator will ensure that data collected conform to all established guidelines for coding collection, key entry and verification. Each subject is assigned a unique subject number to assure subject confidentiality. Information forwarded to the FDA, NIH, NCI or other agencies about subjects on this protocol refers to subjects by a coded identifier and not by name. Subjects will not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The licensed medical records department, affiliated with the institution where the subject receives medical care, maintains all original inpatient and outpatient chart documents.

The research team will maintain Case Report Forms (CRFs) and associated research documentation for each patient treated under the protocol. This documentation includes both clinical data and study-specific documents for each patient. The Principal Investigator or a designee will verify completed CRFs against source documentation on an ongoing basis as they are completed for individual subjects. Data required for analysis of subjects treated on this protocol will be maintained in a password-protected study-specific database. Data from the CRFs are keyed directly into the database by authorized research staff and verified on an ongoing basis.

15.0 TERMINATION OF STUDY

The study will terminate after the last treated patient has completed 90 days of follow-up following liso-cel infusion. CRS and ICANS will be evaluated until day 28 post liso-cel treatment. Disease restaging should be performed approximately on day 28 and day 90. Day-90 disease restaging may be performed

by the patient's local oncologist The study team will contact the local oncologist office by a letter/phone call to request the results of the day-90 restaging, if completed as per standard-of-care.

The PI may terminate the study at any time. The IRB and FDA also have the authority to terminate the study should it be deemed necessary.

16.0 REFERENCES

1. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-Cell Therapy in Refractory Large B-Cell Lymphoma. *N Engl J Med*. 2017;377(26):2531-2544.
2. Hirayama AV, Gauthier J, Hay KA, et al. The response to lymphodepletion impacts PFS in patients with aggressive non-Hodgkin lymphoma treated with CD19 CAR T cells. *Blood*. 2019;133(17):1876-1887.
3. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. *N Engl J Med*. 2019;380(1):45-56.
4. Jacobson CA, Hunter BD, Redd R, et al. Axicabtagene Ciloleucel in the Non-Trial Setting: Outcomes and Correlates of Response, Resistance, and Toxicity. *J Clin Oncol*. 2020;38(27):3095-3106.
5. Nastoupil LJ, Jain MD, Feng L, et al. Standard-of-Care Axicabtagene Ciloleucel for Relapsed or Refractory Large B-Cell Lymphoma: Results From the US Lymphoma CAR T Consortium. *J Clin Oncol*. 2020;38(27):3119-3128.
6. Wang M, Munoz J, Goy A, et al. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. *N Engl J Med*. 2020;382(14):1331-1342.
7. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. 2020;396(10254):839-852.
8. Nastoupil LJ JM, Spiegel JY, et al. . Axicabtagene ciloleucel (axi-cel) CD19 chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory large B-cell lymphoma: real world experience. . *Blood*. 2018;132:91.
9. Jacobson CA HB, Armand P, et al. Axicabtagene ciloleucel in the real world: outcomes and predictors of response, resistance, and toxicity. *Blood* 2018;132:92.
10. Company KPaG. Yescarta and Tecartus Risk Evaluation and Mitigation Strategies; 2020.
11. Novartis. Kymriah Risk Evaluation and Mitigation Strategies; 2020.
12. Turtle CJ, Hanafi LA, Berger C, et al. CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients. *J Clin Invest*. 2016;126(6):2123-2138.
13. Turtle CJ, Hanafi LA, Berger C, et al. Immunotherapy of non-Hodgkin's lymphoma with a defined ratio of CD8+ and CD4+ CD19-specific chimeric antigen receptor-modified T cells. *Sci Transl Med*. 2016;8(355):355ra116.
14. Grupp SA, Kalos M, Barrett D, et al. Chimeric antigen receptor-modified T cells for acute lymphoid leukemia. *N Engl J Med*. 2013;368(16):1509-1518.
15. Davila ML, Riviere I, Wang X, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. 2014;6(224):224ra225.
16. Gust J, Hay KA, Hanafi LA, et al. Endothelial Activation and Blood-Brain Barrier Disruption in Neurotoxicity after Adoptive Immunotherapy with CD19 CAR-T Cells. *Cancer Discov*. 2017;7(12):1404-1419.
17. Nellan A, McCully CML, Cruz Garcia R, et al. Improved CNS exposure to tocilizumab after cerebrospinal fluid compared to intravenous administration in rhesus macaques. *Blood*. 2018;132(6):662-666.
18. Nishimoto N, Terao K, Mima T, Nakahara H, Takagi N, Kakehi T. Mechanisms and pathologic significances in increase in serum interleukin-6 (IL-6) and soluble IL-6 receptor after administration of an anti-IL-6 receptor antibody, tocilizumab, in patients with rheumatoid arthritis and Castleman disease. *Blood*. 2008;112(10):3959-3964.

19. Fujimoto K, Ida H, Hirota Y, Ishigai M, Amano J, Tanaka Y. Intracellular Dynamics and Fate of a Humanized Anti-Interleukin-6 Receptor Monoclonal Antibody, Tocilizumab. *Mol Pharmacol*. 2015;88(4):660-675.
20. Dinarello CA. Biologic basis for interleukin-1 in disease. *Blood*. 1996;87(6):2095-2147.
21. Andersson J, Bjork L, Dinarello CA, Towbin H, Andersson U. Lipopolysaccharide induces human interleukin-1 receptor antagonist and interleukin-1 production in the same cell. *Eur J Immunol*. 1992;22(10):2617-2623.
22. Ohlsson K, Bjork P, Bergenfeldt M, Hageman R, Thompson RC. Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature*. 1990;348(6301):550-552.
23. Jenkins JK, Arend WP. Interleukin 1 receptor antagonist production in human monocytes is induced by IL-1 alpha, IL-3, IL-4 and GM-CSF. *Cytokine*. 1993;5(5):407-415.
24. Carter DB, Deibel MR, Jr., Dunn CJ, et al. Purification, cloning, expression and biological characterization of an interleukin-1 receptor antagonist protein. *Nature*. 1990;344(6267):633-638.
25. Eisenberg SP, Evans RJ, Arend WP, et al. Primary structure and functional expression from complementary DNA of a human interleukin-1 receptor antagonist. *Nature*. 1990;343(6256):341-346.
26. Arend WP, Welgus HG, Thompson RC, Eisenberg SP. Biological properties of recombinant human monocyte-derived interleukin 1 receptor antagonist. *J Clin Invest*. 1990;85(5):1694-1697.
27. Fischer E, Marano MA, Barber AE, et al. Comparison between effects of interleukin-1 alpha administration and sublethal endotoxemia in primates. *Am J Physiol*. 1991;261(2 Pt 2):R442-452.
28. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med*. 2018;24(6):731-738.
29. Norelli M, Camisa B, Barbiera G, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med*. 2018;24(6):739-748.
30. Shah NN, Highfill SL, Shalabi H, et al. CD4/CD8 T-Cell Selection Affects Chimeric Antigen Receptor (CAR) T-Cell Potency and Toxicity: Updated Results From a Phase I Anti-CD22 CAR T-Cell Trial. *J Clin Oncol*. 2020;38(17):1938-1950.
31. Strati P, Ahmed S, Kebriaei P, et al. Clinical efficacy of anakinra to mitigate CAR T-cell therapy-associated toxicity in large B-cell lymphoma. *Blood Adv*. 2020;4(13):3123-3127.
32. Locke FL, Rossi J, Xue X, et al. Abstract CT020: Immune signatures of cytokine release syndrome and neurologic events in a multicenter registrational trial (ZUMA-1) in subjects with refractory diffuse large B cell lymphoma treated with axicabtagene ciloleucel (KTE-C19). *Cancer Research*. 2017:CT020-CT020.
33. Fox E, Jayaprakash N, Pham TH, et al. The serum and cerebrospinal fluid pharmacokinetics of anakinra after intravenous administration to non-human primates. *J Neuroimmunol*. 2010;223(1-2):138-140.
34. Gueorguieva I, Clark SR, McMahon CJ, et al. Pharmacokinetic modelling of interleukin-1 receptor antagonist in plasma and cerebrospinal fluid of patients following subarachnoid haemorrhage. *Br J Clin Pharmacol*. 2008;65(3):317-325.
35. Fisher CJ, Jr., Dhainaut JF, Opal SM, et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. *JAMA*. 1994;271(23):1836-1843.
36. Antin JH, Weinstein HJ, Guinan EC, et al. Recombinant human interleukin-1 receptor antagonist in the treatment of steroid-resistant graft-versus-host disease. *Blood*. 1994;84(4):1342-1348.

37. Antin JH, Weisdorf D, Neuberg D, et al. Interleukin-1 blockade does not prevent acute graft-versus-host disease: results of a randomized, double-blind, placebo-controlled trial of interleukin-1 receptor antagonist in allogeneic bone marrow transplantation. *Blood*. 2002;100(10):3479-3482.
38. McCarthy PL, Jr., Williams L, Harris-Bacile M, et al. A clinical phase I/II study of recombinant human interleukin-1 receptor in glucocorticoid-resistant graft-versus-host disease. *Transplantation*. 1996;62(5):626-631.
39. Emsley HC, Smith CJ, Georgiou RF, et al. A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients. *J Neurol Neurosurg Psychiatry*. 2005;76(10):1366-1372.
40. Singh N, Hopkins SJ, Hulme S, et al. The effect of intravenous interleukin-1 receptor antagonist on inflammatory mediators in cerebrospinal fluid after subarachnoid haemorrhage: a phase II randomised controlled trial. *J Neuroinflammation*. 2014;11:1.
41. Granowitz EV, Porat R, Mier JW, et al. Pharmacokinetics, safety and immunomodulatory effects of human recombinant interleukin-1 receptor antagonist in healthy humans. *Cytokine*. 1992;4(5):353-360.
42. Yang BB, Gozzi P, Sullivan JT. Pharmacokinetics of Anakinra in Subjects of Heavier vs. Lighter Body Weights. *Clin Transl Sci*. 2019;12(4):371-378.
43. Park JH, Sauter CS, Palomba ML, et al. A Phase II Study of Prophylactic Anakinra to Prevent CRS and Neurotoxicity in Patients Receiving CD19 CAR T Cell Therapy for Relapsed or Refractory Lymphoma. *Blood*. 2021;138:96.
44. Monteagudo LA, Boothby A, Gertner E. Continuous Intravenous Anakinra Infusion to Calm the Cytokine Storm in Macrophage Activation Syndrome. *ACR Open Rheumatol*. 2020;2(5):276-282.
45. Lee DW, Santomasso BD, Locke FL, et al. ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. *Biol Blood Marrow Transplant*. 2019;25(4):625-638.
46. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. 2014;124(2):188-195.
47. Cheson BD, Fisher RI, Barrington SF, et al. Recommendations for initial evaluation, staging, and response assessment of Hodgkin and non-Hodgkin lymphoma: the Lugano classification. *J Clin Oncol*. 2014;32(27):3059-3068.
48. Association FaD. Approved Risk Evaluation and Mitigation Strategies (REMS) Breyanzi (lisocabtagene maraleucel); 2021.
49. Zhou H, Lee JJ, Yuan Y. BOP2: Bayesian optimal design for phase II clinical trials with simple and complex endpoints. *Stat Med*. 2017;36(21):3302-3314.

APPENDIX A: ASTCT Cytokine Release Syndrome (CRS) Consensus Grading[45]

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever[1]	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C
With				
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or[2]				
Hypoxia	None	Requiring low-flow nasal cannula[3] or blow-by	Requiring high-flow nasal cannula[3], facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (e.g.,, CPAP, BiPAP, intubation and mechanical ventilation)

Organ toxicities associated with CRS may be graded according to CTCAE v5.0 but they do not influence CRS grading.

[1] Fever is defined as temperature ≥38°C not attributable to any other cause. In patients who have CRS then receive antipyretic or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS grading is driven by hypotension and/or hypoxia.

[2] CRS grade is determined by the more severe event: hypotension or hypoxia not attributable to any other cause. For example, a patient with temperature of 39.5°C, hypotension requiring 1 vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade 3 CRS.

[3] Low-flow nasal cannula is defined as oxygen delivered at ≤6L/minute. Low flow also includes blow-by oxygen delivery, sometimes used in pediatrics. High-flow nasal cannula is defined as oxygen delivered at >6L/minute.

APPENDIX B. 2014 NIH CRS Grading ("Lee criteria")

Grade	Toxicity
Grade 1	Symptoms are not life threatening and require symptomatic treatment only, e.g., fever, nausea, fatigue, headache, myalgias, malaise
Grade 2	Symptoms require and respond to moderate intervention Oxygen requirement <40% or Hypotension responsive to fluids or low dose of one vasopressor or Grade 2 organ toxicity per CTCAE 4.0
Grade 3	Symptoms require and respond to aggressive intervention Oxygen requirement ≥40% or Hypotension requiring high dose or multiple vasopressors or Grade 3 organ toxicity or grade 4 transaminitis
Grade 4	Life-threatening symptoms Requirement for ventilator support or Grade 4 organ toxicity (excluding transaminitis)
Grade 5	Death

Criteria defining high-dose vasopressor use	
Vasopressor	**Dose**
Norepinephrine monotherapy	≥ 20 μg/min
Dopamine monotherapy	≥ 10 μg/min
Phenylephrine monotherapy	≥ 200 μg/min
Epinephrine monotherapy	≥ 10 μg/min
If on vasopressin	Vasopressin + norepinephrine equivalent of ≥ 10μg/min*
If on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥20μg/min

*VASST Trial vasopressor equivalent equation: norepinephrine equivalent dose = [norepinephrine (μg/min)] + [dopamine (μg/kg/min) 2] + [epinephrine (μg/min)] + [phenylephrine (μg/min) 10].

APPENDIX C: ASTCT ICANS* Consensus Grading[45]

* Immune Effector Cells (IEC)-associated neurotoxicity syndrome (ICANS)

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score[1, 2]	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness[3]	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between
Motor findings[4]	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/cerebral edema	N/A	N/A	Focal/local edema on neuroimaging[5]	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause; for example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS.
N/A indicates not applicable.

[1] ICE Score:

- **Orientation:** orientation to year, month, city, hospital; 4 points
- **Naming:** ability to name 3 objects (e.g. point to clock, pen, button): 3 points
- **Following commands:** ability to follow simple commands (e.g., “show me 2 fingers” or “close your eyes and stick out your tongue”): 1 point
- **Writing:** ability to write a standard sentence (e.g. “Our national bird is the bald eagle”): 1 point
- **Attention:** ability to count backwards from 100 by 10: 1 point

[2] A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.

[3] Depressed level of consciousness should be attributable to no other cause (e.g., no sedating medication).

[4] Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.

[5] Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

APPENDIX D: Schedule of Assessments

Assessment	Pre-Lymphodepletion Evaluations	Day of liso-cel Infusion (Day 0)	Follow-up Evaluations									
			1	3	7	10	13	14	21	28	90	Comments
I/E criteria	X											
Medical history	X	X	X		X			X	X	X		
Physical exam	X	X	X		X			X	X	X		
ICE score		X	X	X	X	X		X	X	X		
Height/weight	X											
Karnofsky performance status	X											
CRS/ICANS-related interventions		Continuous recording from day 0-28										
AEs		Continuous recording from day 0-28										
Vitals including pulse oximetry	X	Prior to and 30 minutes after anakinra administration on days 0 through 13										For patients receiving step-up dosing, vital signs will be obtained every 4 hours per standard-of-care.
Chest x-ray	X											
12-lead ECG	X											
MUGA/ECHO	X											
CT/PET or CT	X									X	X	As clinically indicated
BMA and biopsy	X									X	X	As clinically indicated
Lumbar puncture	X									X	X	As clinically indicated
Lymphodepleting chemotherapy												Lymphodepleting chemotherapy per

Assessment	Pre-Lymphodepletion Evaluations	Day of liso-cel Infusion (Day 0)	Follow-up Evaluations									
			1	3	7	10	13	14	21	28	90	Comments
												institutional practice
Liso-cel administration		X										liso-cel infusion per institutional practice
Anakinra administration		day 0-13										
Laboratory evaluations												
Pregnancy test	X											
CBC, differential	X	X	X	X	X	X		X	X	X		
Renal/Hepatic Function Panel	X	X	X	X	X	X		X	X	X		
Uric acid	X	X	X	X	X	X		X	X	X		
Serum ferritin	X	X	X	X	X	X		X	X	X		
CRP	X	X	X	X	X	X		X	X	X		
IL-6	X	X	X	X	X	X		X	X	X		
PT, PTT, fibrinogen, thrombin time and D-dimer	X	X	X	X	X	X		X	X	X		
Quantitative serum IgG level	X											
Triglycerides	X	X	X	X	X	X		X	X	X		
PK samples		X[1]	X[2]									

[1]Approximately 4 hours after the end of the first anakinra administration
[2]Approximately 24 hours (+/- 1 hour) after the end of the first anakinra administration and prior to the second anakinra administration
The proposed days of all assessments and laboratory evaluations are approximate and may vary due to scheduling, clinical factors, research priorities or other considerations. All timepoints may not be collected. Laboratory research evaluations may vary according to clinical and research priorities.