Masonic Cancer Center, University of Minnesota
Cancer Experimental Therapeutics Initiative (CETI)

Intraperitoneal FATE FT516 and Interleukin-2 (IL-2) with Intravenous Enoblituzumab in Recurrent Ovarian, Fallopian Tube, and Primary Peritoneal Cancer

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Revision History

Revision #	Version Date	Detail of Changes	Consent Change?
	Mar 16 2020	Original version for FDA	n/a
	Apr 27 2020	In response to FDA Clinical review received by email on Apr 22 2020 (IND serial 0004) Other interim updates: • Add MacroGenics assigned study number to title page • Remove in-line filter requirement for FT516 as filter is only required as a safety precaution for IV infusion • Refine grade 3 organ toxicity definition within DLT definition • Section 5.1.8 clarify washout period of a least 14 days only applies to SOC tumor directed therapy to make consistent with Section 5.2.5 • Add UMN study number for LTFU study • Section 7.4 – add paragraph regarding decision to treat on day of planned FT516 • Section 8.5 – clarify pre-meds for FT516 also serve as pre-meds for IL-2 • Section 9.1 and Section 9.2 – add a new column for Prior to 1st dose of study drug (baseline) • Section 9.2 - add PAR anti-HLA antibodies at baseline and Day 36	yes
	Apr 29 2020	In In response to FDA Clinical review received by email on Apr 28 2020 (IND serial 0005) – FDA approved version Other interim updates: Section 10.5: Advarra is the IRB of record, not UMN IRB Section 8.3 – edit DMSO reaction management section as not expected with low cell volume via IP infusion.	yes
	Jun 22 2020	Original to CPRC after MacroGenics review Other minor clarification and edits	no
	Jul 15 2020	In response to minor CPRC stip – Original to IRB Section 13.4 Update stopping rule language. Section 10.5 – clarify MacroGenics is only cc'ed on FDA submission for patients receiving enoblituzumab (Cohort 4 and 5)	no
1	Oct 27 2020	• Clarify after CCPM throughout protocol including synopsis, schema, Section 4, Section 7.5 – study design that a minimum of 28 days between patient cohorts (end of DLT) and 14 days between the 1st and 2nd patients within a cohort and rules of enrollment including situations where Stage 1 Step 2 or Stage 2 are not activated. Re write for clarity • Synopsis –move rationale section down in synopsis to keep Study Design on one page • Synopsis and Section 5.1.6 - Update inclusion criteria for the placement of the IP catheter to read before the 1st dose of study directed drug as enoblituzumab is given before LD chemo.	yes

Revision #	Version Date	Detail of Changes	Consent Change?
		• Synopsis and Section 5.2.1 add exclusion of women who are pregnant or plan on becoming pregnant in the next 6 months • Section 7.2 – clarify additional doses of eno starts on Day 22, not Day 21 • Section 7.4 – add rules for window from CY/FLU administration of FT516, scheduling issues and skipped doses, match vital signs to wording in footnote in Section 9.1 SOC care table • Section 7.8 and 7.9 – minor edits • Section 7.10 – add a statement that enoblituzumab may continue for Cohorts 4 and 5 • Section 8.1 – clarify in header that IRR is for Cohorts 4 and 5 only • Section 8.5 – update diphenhydramine dose information to match SOC • Section 8.6 – update CRS section regarding collection of IL-6 levels if CRS is suspected or diagnosed. Update Table 2, grade 2 hypotension management with new toci guidelines, add toci PI as ref., update Section 9.1 to reflect clarification • Section 9.1 – update based on updated Section 8.6 • Section 9.2 – update research sample collection including added baseline safety samples for Fate, clarify the 9 month safety samples are not required for Fate, reduce follow-up blood volumes for green top tubes to 30 ml per time point, delete PB and IP chimerisms, added new footnote for part of a green top tube to go to Fate to replace chimerisms run locally, other minor clarifications • Section 10.2 – add standard template language • Update Section 12.5 – record retention to new template langue • Other minor updates and clarifications	
2	Jan 11 2022	• Section 7.1 – add the of risk for spread of cancer cells that are located within the peritoneum to surrounding tissues with placement and use of IP catheter • Section 10.5 - update Advarra and Fate expedited reporting timeframes • Section 6.3, Section 7.8 - Clarify retreatment criteria of stable disease or better is based on iRECIST (iCR, iPR or iSD) not RECIST (CR, PR, SD) and if a iUPD at the Day 36 disease assessment, options regarding retreatment • Updated table of Key Abbreviations	yes

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Key Abbreviations

Abbreviation	Definition
ABW	actual body weight
ADCC	antibody dependent cell-mediated cytotoxicity
ADL	activities of daily living
AE	adverse event
ASTCT	American Society for Transplantation and Cellular Therapy
CAR	chimeric antigen receptor
CFR	Code of Federal Regulations
CNS	central nervous system
CRM	continual reassessment method
CRS	cytokine release syndrome
CTCAE	Common Terminology Criteria for Adverse Events
CY/FLU	cyclophosphamide/fludarabine
DLCO	diffusing capacity of the lungs for carbon monoxide
DLT	dose limiting toxicity
eCRF	electronic case report form
EOT	end of treatment
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GvHD	graft-versus-host disease
IB	Investigator's Brochure
ICANS	Immune effector Cell-Associated Neurotoxicity Syndrome
ICE	Immune Effector Cell-Associated Encephalopathy
ICH	International Conference on Harmonisation
iCPD	confirmed progressive disease based on iRECIST
iCR	complete response based on iRECIST
IL-2	Interleukin-2
IND	Investigational New Drug
IP	Intraperitoneal
iPR	partial response based on iRECIST
IRB	Institutional Review Board
iRECIST	Immune Response Evaluation Criteria in Solid Tumors
IRR	Infusion related reaction
iSD	stable disease based on iRECIST
iUPD	unconfirmed progressive disease based on iRECIST
IV	intravenous
LTFU	long-term follow-up
MTD	maximum tolerated dose
NCI	National Cancer Institute
NK	natural killer
OS	overall survival
PBMC	peripheral blood mononuclear cell
PFS	progression free survival
PFT	pulmonary function test
SAE	serious adverse event
SOC	Standard of care
SUSAR	Suspected Unexpected Serious Adverse Reaction
TRM	treatment related mortality
ULN	upper limit of normal

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Protocol Synopsis

Intraperitoneal FATE FT516 and Interleukin-2 (IL-2) with Intravenous Enoblituzumab in Recurrent Ovarian, Fallopian Tube, and Primary Peritoneal Cancer

Study Design:

This is a single center Phase I clinical trial of FT516 administered intraperitoneally (IP) once a week for 3 consecutive weeks for the treatment of recurrent gynecologic cancers. As this is an early 1st in human study and the 1st intraperitoneal infusion of FT516, the safety of FT516 is confirmed prior to adding enoblituzumab as an intravenous infusion approximately 1 week prior to the 1st dose of FT516 and every 3 weeks beginning on Day 22 (1 week after the last dose of FT516). Each dose of FT516 is followed directly by an IP infusion of interleukin-2 (IL-2) to facilitate natural killer (NK) cell survival. A short course of outpatient lymphodepletion chemotherapy is given prior to the 1st dose of FT516.

FT516 is an off the shelf product comprised of allogeneic natural killer (NK) cells, expressing high-affinity non-cleavable CD16 (FT516). Enoblituzumab is an Fc-optimized monoclonal antibody that targets B7-H3 which is highly expressed on ovarian cancer.

This study is conducted in two consecutive stages using the following treatment plan:

Cohort	Treatment Plan
1 (start)	IP FT516 monotherapy 9 x 10^7 cells/dose on Day 1, 8, and 15
2	IP FT516 monotherapy 3 x 10^8 cells/dose on Day 1, 8, and 15
3	IP FT516 monotherapy 9 x 10^8 cells/dose on Day 1, 8, and 15
4	Safe dose (MTD-1) from 1st 3 levels + IV enoblituzumab on Day -6
5	Highest dose (MTD) from 1st 3 levels + IV enoblituzumab on Day -6

A minimum of 28 days must separate each cohort. Within a 3 patient cohort, a minimum of 14 days must separate the 1st and 2nd patient within a cohort. All patients are assessed for Dose Limiting Toxicity (DLT) as defined in the Schema and in Section 7.5

Stage 1 Step 1 uses a fast-track design (1 patient per Cohort) with a minimum of 28 days between each patient until one of the following:

- The 1st occurrence of a pre-defined adverse event within 28 days of the cell infusion (defined as Grade 3 abdominal pain for > 48 hours despite standard analgesics or Grade 3 infusion related reaction) at which point the study moves to **Step 2** and the cohort size increases from 1 to 3 patients with 2 additional patients added to the current cohort. Escalation in Step 2 continues until the 1st DLT event at which point Stage 2 (CRM) is activated. If Cohort 5 is completed with 10 patients enrolled at the MTD without a DLT, Stage 2 (CRM) is not used.
- The 1st occurrence of a DLT the study moves directly to Stage 2 (CRM) as detailed below and Step 2 is not used.
- Cohort 5 is completed without a pre-defined AE or a DLT – neither Step 2 nor Stage 2 is used if a total of 10 patients are enrolled in Cohort 5 to complete the study.

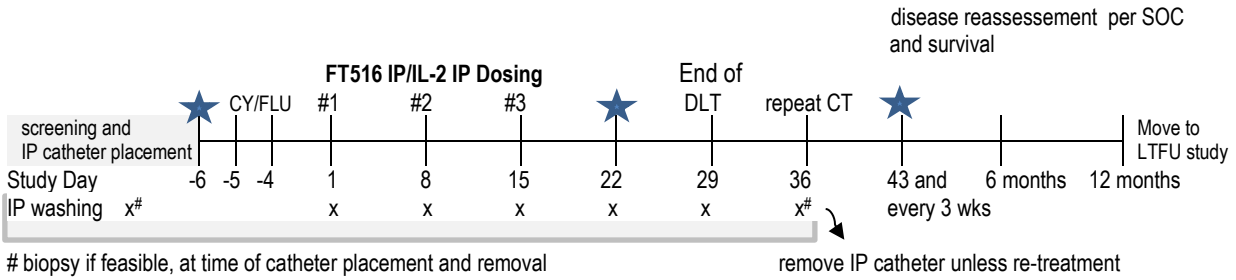
Stage 2 is initiated at the 1st DLT (as defined in Schema and in Section 7.5**)**. The study design changes to the continual reassessment method (CRM). Enrollment occurs in cohorts of three with a minimum of 14 days between the 1st and 2nd patient. Each new cohort of three patients are sequentially assigned to the most appropriate dose by the study statistician based on the updated toxicity probabilities once the 3rd patient in a cohort reaches Day 28 (end of DLT period). The MTD will be identified by the minimum of the following criteria: (1) the total Stage 2 sample size of 25 is exhausted, (2) 10 consecutive patients are enrolled at the same dose plan or (3) the probability that the next 5 patients will be allocated to the same dose plan, based on the current estimate of the probability of toxicity, exceeds 90%.

For study endpoints, follow-up continues until disease progression and then for survival only for 1 year from the 1st dose of FT516.

Investigational Products:	• Enoblituzumab provided by MacroGenics, Inc. • FT516 provided by Fate Therapeutics • IL-2 paid for by the study (research grants)
Rationale for the Combination:	Based on data showing that within the ovarian cancer tumor microenvironment surface expression of CD16a on NK cells is diminished, we hypothesize that the FT516 cellular product containing a non-cleavable CD16 will bypass the low CD16 expression issue and maximize NK cell cytotoxicity. Enoblituzumab is an Fc optimized humanized IgG1 monoclonal antibody that binds to B7-H3 (CD276). B7-H3 is an inhibitory immune checkpoint molecule that is widely expressed by a number of different tumor types and may play a key role in regulating the immune response. It is therefore hypothesized that the combination of FT516 with enoblituzumab will maximize NK cell cytotoxicity in patients with ovarian cancer.
Long-Term Follow-Up:	After 1 year, follow-up transfers to a separate long-term follow-up (LTFU) study (CPRC #2020LS072) to continue the FDA's required 15-year follow-up after treatment with a genetically modified cell therapy. Participation in the LTFU study is mandatory.
Primary Objective:	To determine the maximum tolerated dose (MTD) of FT516 monotherapy when administered via intraperitoneal catheter with IP IL-2 and in combination with intravenous (IV) enoblituzumab in patients with recurrent ovarian, fallopian tube, and primary peritoneal cancer.
Secondary Objectives:	• To characterize the toxicities associated with IP FT516 and IL-2 when administered as monotherapy and after IV enoblituzumab • To estimate progression-free survival (PFS) and overall survival (OS) at 6 months and 12 months from the 1st dose of FT516 • To gain preliminary efficacy information with this treatment combination
Correlative Objectives:	Blood and intraperitoneal washings: • To characterize the pharmacokinetics (PK) of FT516 monotherapy and in combination with enoblituzumab in the peritoneal fluid, peripheral blood and tumor biopsy samples. • To assess the association of PK and pharmacodynamics (PD) in the peritoneal fluid of FT516 monotherapy and in combination with enoblituzumab in the peritoneal fluid in recurrent ovarian, fallopian tube, and primary peritoneal cancer with safety and anti-tumor activity • To measure function of in vivo expanded adoptively transferred FT516 Tumor biopsies: • To assess tumor microenvironment
Inclusion Criteria:	• Recurrent epithelial ovarian cancer, fallopian tube, or primary peritoneal cancer meeting one of the following minimal prior treatment requirement (no maximum number of prior treatments): platinum resistant: FT516 may be given as 2nd line (first salvage therapy) platinum sensitive: FT516 may be give as 3rd line therapy (second salvage therapy) • Must have measurable disease per iRECIST1.1 within the abdomen and pelvis assessed within 42 days of the 1st FT516 infusion • Extra-peritoneal disease is permitted; however each lesion must be <5 cm in diameter • Agrees to the placement of an intraperitoneal catheter before the 1st dose of study directed drug (chemotherapy or enoblituzumab - Cohort 4 and 5) and remains in place through Day 36 or longer • ≥ 18 years of age at time of consent signing • GOG performance status ≤ 2 • Adequate organ function • Provides written consent to the companion LTFU study CPRC#2020LS072 • Voluntary written consent for this study

Exclusion Criteria:	• Pregnant or planning on becoming pregnant in the next 6 months • Known allergy to the following FT516 components: albumin (human) or DMSO • Any known condition that requires systemic immunosuppressive therapy (> 5mg prednisone daily or equivalent) during the FT516 dosing period (3 days before the 1st dose through 14 days after the last dose) excluding pre-medications – inhaled and topical steroids are permitted • Receipt of any investigational agent within 28 days prior to the first dose of investigational product • Known seropositive for HIV or known active Hepatitis B or C infection with detectable viral load by PCR
Accrual:	Between 14 and 31 patients based on toxicity (includes 10 patients treated at the MTD)

Schema



Enoblituzumab 15 mg/kg IV 1 day before lymphodepleting chemotherapy (LEVEL 4 and LEVEL 5 only)

CY/FLU Lymphodepleting Chemo: Fludarabine 25 mg/m^2 IV followed by CY 300 mg/m^2 IV) given on 2 consecutive days with a minimum of 48 hours between the last dose of Flu and 1st dose of FT516 (e.g. day -5, day-4)

FT516 IP at assigned dose* followed directly by **IL-2 IP** 6 MIU on Days 1, 8, and 15

Enoblituzumab 15 mg/kg IV continues once every 3 weeks beginning on Day 22 and continuing every 3 weeks until disease progression or unacceptable toxicity. (LEVEL 4 and LEVEL 5 only)

Day 29: End of DLT assessment period
Day 36: Disease reassessment with CT of chest/abd/pelvis – remove catheter if no retreatment or if retreatment is planned administer in an identical schedule to with CY/FLU to start 1-2 days after next planned enoblituzumab (on Day 43).

Follow-up: for disease response until disease progression and then survival only per standard of care for 1 year from the 1st dose of FT516

***FT516 Dose Level Assignment**

Cohort	FT516 Dose IP (cells per dose)	Enrollment Plan - A minimum of 28 days must separate each cohort. Within a 3 patient cohort, a minimum of 14 days must separate the 1st and 2nd patient within a cohort. All patients are assessed for Dose Limiting Toxicity (DLT) as defined below and in Section 7.5
1	Monotherapy: IP FT516 at 9 x 10^7 cells/dose on Day 1, 8, and 15	• **Stage 1, Step 1**: Begin at Level 1. Enroll one patient per level until one of the following occurs within 28 days of 1st FT516 dose: Abdominal pain that is unresponsive to standard analgesics lasting more than 48 hours not attributable to disease status OR Grade 3 infusion related reaction (per CTCAE V5) OR 1st DLT as defined below. **Note:** if a DLT occurs during Fast-track (Step 1), enrollment moves directly to Stage 2 CRM and Step 2 is not used. If Cohort 5 is completed (N=10) without a pre-defined AE or a DLT – neither Step 2 nor Stage 2 is used. • **Stage 1, Step 2**: Increase patient cohorts to 3 and continue sequential dose escalation in cohort of 3 patients while assessing for DLT as defined below. At the 1st DLT switch to Stage 2. If Cohort 5 is completed (N=10) without a DLT, Stage 2 (CRM) is not used. • **At the 1st DLT, Stage 2** is initiated and enrollment continues using the continual reassessment method (CRM), enrolling cohorts of 3 patients at the most appropriate level as determined by the study statistician once the 3rd patient in a cohort reaches Day 28 (end of DLT period) until 25 patients are enrolled by CRM or 10 consecutive patients are enrolled at the same level or the probability that the next 5 patients will be allocated to the same level based on the current estimate of the probability of toxicity, exceeds 90%.
2	Monotherapy: IP FT516 at 3 x 10^8 cells/dose on Day 1, 8, and 15	
3	Monotherapy: IP FT516 at 9 x 10^8 cells/dose on Day 1, 8, and 15	
4	Safe FT516 dose (MTD-1) from 1st 3 levels + IV enoblituzumab on Day -6	
5	Highest FT516 dose (MTD) from 1st 3 levels + IV enoblituzumab on Day -6	

Dose Limiting Toxicity (DLT) is defined as any treatment emergent toxicity at least possibly related to the study treatment meeting one of the following criteria based on CTCAE v5 within 28 days (within 14 days for ascites) of the 1st FT516 infusion (for Cohort 4 and 5, DLT assessment starts with enoblituzumab and continues for 28 days after 1st FT516):

- Grade 3 organ toxicity (pulmonary, hepatic, renal, or neurologic) not pre-existing and lasting more than 72 hours
- Any non-hematologic Grade 4 or 5 toxicity
- Neutrophil count decreased ≥ Grade 4 that persists at Day 28 despite use of growth factor support
- Grade 3 abdominal pain lasting more than 4 consecutive days and not controlled by standard analgesics
- Grade 3 or greater ascites within 14 days after FT516 administration in patients who had no ascites or Grade 1 ascites at enrollment and is not attributable to disease progression

1 Objectives

1.1 Primary Objective

The primary objective of the study is to determine the maximum tolerated dose (MTD) of FT516 monotherapy when administered via intraperitoneal catheter with IP IL-2 and in combination with intravenous enoblituzumab in patients with recurrent ovarian, fallopian tube, and primary peritoneal cancer.

1.2 Secondary Objectives

- To characterize the toxicities associated with IP FT516 and IL-2 when administered as monotherapy and after IV enoblituzumab.
- To estimate progression-free survival (PFS) and overall survival (OS) at 6 months and 12 months from the 1st dose of FT516.
- To gain preliminary efficacy information with this treatment combination.

1.3 Correlative Objectives

Blood and intraperitoneal washings are collected prior to treatment and at several time points through Day 42 (blood) or Day 36 (peritoneal fluid) for study related analysis:

- To characterize the pharmacokinetics (PK) of FT516 monotherapy and in combination with enoblituzumab in the peritoneal fluid, peripheral blood and tumor biopsy samples.
- To assess the association of PK and pharmacodynamics (PD) in the peritoneal fluid of FT516 monotherapy and in combination with enoblituzumab in recurrent ovarian, fallopian tube, and primary peritoneal cancer with safety and anti-tumor activity.
- To measure function of in vivo expanded adoptively transferred FT516.

Tumor biopsies (if feasible) at the time of catheter placement and catheter removal for study related analysis including:

- To assess tumor microenvironment.

2 Background and Significance

2.1 Background

Dr. Jeffrey Miller and collaborators from the University of Minnesota pioneered the development of allogeneic haplo-identical natural killer (NK) cell cancer immunotherapy beginning in the year 2000. While previous clinical trials of methods to induce autologous NK cell activity demonstrated safety, such as

prolonged treatment with low-dose subcutaneous IL-2, higher doses of IV IL-2 and infusions of ex vivo IL-2 activated NK cells, these methods failed to demonstrate clinical efficacy. As the concept of "missing self" and the rules of NK cell alloreactivity emerged, interest in the use of haplo-identical NK cells to increase anti-tumor activity was established. It was believed that effector NK cells educated in haplo-identical healthy donors could induce stronger graft-versus-leukemia effects since these cells were not exposed to immunosuppressive mechanisms found in cancer patients. Additionally, it was further hypothesized that haplo-identical donors would provide a higher frequency of alloreactive NK cells (i.e., NK cells with attenuated inhibitory receptor signaling due to the major histocompatibility complex class I mismatch between the haplo-identical NK cells and the patient's tumor cells).

We established the safety and success of adoptive transfer of allogeneic NK cell products in a trial using haplotype mismatched, related-donor NK cell products (mean NK cell dose of 2 (range 1-6.2) $x10^7$ cells/kg from a single donor apheresis), followed by subcutaneous IL-2 to induce in vivo NK survival and expansion (Miller 2005). Importantly, successful expansion of the allogeneic NK cells was achieved after a lympho-depleting regimen of high dose cyclophosphamide (Cy) and fludarabine (Flu). This chemotherapy regimen, delivered prior to NK cell transfer, transiently prevents patient T cells from rejecting adoptively transferred NK cells creating an environment conducive to NK cell expansion. Complete remissions in AML correlated with in vivo NK expansion and higher proportions of circulating (and functional) NK cells. This treatment approach leads to potent anti-tumor killing. To date, we have treated over 150 patients with refractory AML and other cancers, including ovarian, with adoptively transferred NK cells (INDs 8847, 13659,14448) utilizing our standard platform of Cy/Flu, followed by delivery of exogenous IL-2 or IL-15 to promote in vivo NK cell activation and expansion. Although these results are an improvement over standard salvage therapy for patients with refractory leukemia (expected CR rate of 30% with NK therapy vs 10% with current SOC), they suggest that adoptive cell therapy in its current form requires additional anti-tumor activity. (Bachanova 2014)

2.2 Natural Killer (NK) Cells

2.2.1 Natural Killer Cells as Immunotherapy

Cancer immunotherapy is a rapidly evolving field that has transformed the treatment of many tumor types including solid tumors. However, despite important advances, the majority of patients will either not respond or eventually experience disease relapse. Particularly in solid tumors, the mechanisms of tumor resistance

to cancer immunotherapies are diverse and include the ability of tumors to form physical and immunologic barriers to immune effector cells such as T cells and NK cells (Melero 2014). Further understanding of the biology that enables these cells to enter tumors and retain anti-tumor cytotoxic activity is important in order to maximize their clinical benefit for patients.

NK cells are so named for their "natural" ability to kill cancer cells without prior sensitization (Kiessling 1975). NK cells kill cancer cells by multiple mechanisms including direct cytotoxicity, cytokine secretion, and antibody dependent cell-mediated cytotoxicity (ADCC):

- Direct cytotoxicity through the targeted release of perforins and granzymes. Importantly, while MHC-I deficient cells evade CD8 T-cell recognition, they are preferential targets for NK cells and are highly susceptible to NK cell-mediated killing (Malmberg 2017).
- Cytokines, including interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) promote direct tumor cell killing (Wang R 2012).
- ADCC, which occurs when an antibody binds to a tumor cell and the antibody's Fc region binds to the CD16 receptor on NK cells, triggering a cytotoxic response towards the tumor cell (Waldhauer 2008; Wang, W 2015).

In addition to direct effects on tumor cells, NK cells can interact with the adaptive immune system to generate and maintain adaptive immune responses against cancer cells:

- Killing of tumor cells by NK cells results in the release of tumor antigens for recognition by the adaptive immune system (Dahlberg 2015).
- NK cells upon activation secrete cytokines that recruit and activate endogenous T cells. Importantly, activated NK cells are potent producers of chemokines such as CXCL10, CCL4, and CCL5, which are known recruitment factors for T cells. Cytokines secreted by NK cells also induce maturation of dendritic cells, which serve as antigen-presenting cells to mediate adaptive immune responses (Smyth 2002).

2.2.2 Clinical Outcomes of Allogeneic NK Cell Therapy

In clinical investigations, allogeneic NK-cell therapies have been well-tolerated with documented anti-tumor activity. More than 500 patients across 30 completed clinical studies have received allogeneic NK cells (Veluchamy 2017). Notably, and unlike allogeneic T cell therapies, allogeneic NK cells have not been associated with graft-vs-host disease (GvHD). Furthermore, with a single exception (Cooley 2019), allogeneic NK cell therapies have not been associated with cytokine release

syndrome (CRS) or neurotoxicity, common complications observed with CAR-T cell therapies. Complete remission rates ranging from 21% to 53% have been observed following a single administration of allogeneic NK cells in subjects with relapsed/refractory acute myelogenous leukemia (AML) (Miller 2005; Bachanova 2014; Romee 2016), and in subjects with poor prognosis refractory non Hodgkin lymphoma (Bachanova 2018). Clinical responses have also been reported in subjects with solid tumors including non-small cell lung cancer (Iliopoulou 2010; Tonn 2013), as well as in subjects with platinum-resistant ovarian cancer (Geller 2011), melanoma (Arai 2008), and renal cell carcinoma (Arai 2008).

2.3 FT516

2.3.1 Development of FT516

FT516 is an allogeneic natural killer (NK) cell immunotherapy derived from a clonal human-induced pluripotent stem cell (iPSC) line, engineered to express a high-affinity, non-cleavable CD16 (hnCD16) Fc receptor. The clonal master cell bank (MCB) used for the production of FT516 was generated by selecting and expanding a single, well-characterized iPSC clone with a single, defined hnCD16 transgene integration site. The use of a clonal MCB as the starting material for routine Current Good Manufacturing Practices production of FT516 is intended to directly address many of the limitations associated with patient- and donor-specific cell therapies. Notably, many doses of FT516 drug product can be uniformly produced in a single manufacturing campaign. These doses of drug product are homogeneous and can be (i) tested to assure compliance with a pre-defined quality specification, (ii) cryopreserved in an infusion medium, and (iii) stored to maintain a sustainable inventory. As such, in the clinical setting, FT516 has off-the-shelf availability for use in multi-dose regimens, which may prove critical for driving long-term durable responses in patients with progressing disease.

The engineered features of FT516 are designed to result in increased activity against target tumor cells as monotherapy and when combined with monoclonal antibodies (mAbs) that can mediate antibody dependent cell cytotoxicity (ADCC). Important functional attributes of FT516 include the following:

- FT516 is expected to have superior effector function compared to patients' endogenous NK cells, which are diminished in number and poorly functional due to prior treatment regimens (e.g., chemotherapy) and tumor suppressive mechanisms. FT516 maintains the "natural cytotoxicity" that is potent and specific to transformed cells.
- FT516 expresses an hnCD16 Fc receptor. The high-affinity CD16 variant arising from a naturally occurring 158V polymorphism has demonstrated enhanced

ADCC when combined with therapeutic mAbs in nonclinical studies. In clinical studies, patients whose endogenous NK cells express the high affinity CD16 Fc receptor variant, higher objective response rates, and increased progression-free survival (PFS) were observed with treatment with rituximab, cetuximab, and trastuzumab (Cartron 2002; Musolino 2008; Bibeau 2009). In addition, hnCD16 contains the genetic alteration (S179P) that prevents cleavage of CD16 by the metalloproteinase ADAM17 (Lajoie 2014; Jing 2015) that is a mechanism in the regulation of NK cell activity (Romee 2013).

The product candidate is being investigated in an open-label, multi-dose Phase 1 clinical trial as a monotherapy for the treatment of acute myeloid leukemia and in combination with CD20-directed monoclonal antibodies for the treatment of advanced B-cell lymphoma (NCT04023071). Subjects are eligible to receive up to three once-weekly doses over a one-month treatment cycle, which treatment cycle may be repeated. FT516 cells are formulated in an infusion medium consisting of Plasma-Lyte A (pH 7.4) with albumin (human) (10% w/v) and dimethyl sulfoxide (7.5% v/v), and is administered intravenously.

2.3.2 Summary of Clinical Data

A Phase I study of FT516 in subjects with advanced hematologic malignancies is ongoing. Enrolled subjects may receive up to a total of six doses of FT516 by intravenous (IV) infusion, each cycle of three doses preceded by low dose chemotherapy and followed by supplemental IL-2. As of the Development Safety Update Report (DSUR) data cutoff date of 31 January 2020, three subjects have been treated with FT516 in the Phase I study, with two subjects treated with Regimen A (FT516 IV monotherapy) and one subject treated with Regimen B (FT516 IV plus rituximab) as follows:

Regimen	**NK Cells per Dose**	**Number of Subjects**	**Total Number of FT516 Doses [1]**
Regimen A: FT516	9×10^7 cells	2	9
Regimen B: FT516 + rituximab	3×10^7 cells	1	6

[1] Total number of FT516 IV doses represents the cumulative number of doses administered to all subjects enrolled at the prescribed FT516 dose. As of the DSUR data cutoff date, enrolled subjects each received between 3 and 6 doses of FT516: Regimen A (9×10^7 cells) – 1 subject received 6 doses and 1 subject received 3 doses; Regimen B (3×10^7 cells) – 1 subject received 6 doses.

As of the DSUR data cutoff date, no serious clinical risks related to intravenous FT516 had been identified. No subjects had experienced cytokine release syndrome (CRS), neurotoxicity or graft vs. host disease. In Regimen A, no dose-limiting toxicities (DLTs), or FT516-related serious adverse events (SAEs), were reported. In Regimen B, a protocol-defined dose-limiting toxicity (DLT) of Grade 4 neutropenia in the absence of fever or signs and symptoms of infection was reported; however, multiple factors other than FT516, including baseline clinical factors, effects of lympho-conditioning and the known toxicity profile of rituximab, may have contributed to the neutropenia.

2.3.3 Summary of Nonclinical Data

Nonclinical *in vitro* and *in vivo* studies were conducted using cryopreserved FT516-R, the non-GMP research use equivalent of FT516. No safety concerns were identified. Data from nonclinical studies were notable for the following:

- No safety concerns have been observed. A three-month Good Laboratory Practice (GLP) toxicology study revealed no FT516-R related serologic or histologic evidence of toxicity with repeated dosing at doses calculated to be 26-times higher than the proposed clinical starting dose based on allometric scaling. Furthermore, no evidence of tumorigenicity was identified.
- The biodistribution of FT516-R cells is consistent with the expected tissue distribution following intravenous (IV) administration of adoptive cells, predominantly in highly perfused tissues such as lungs, spleen, heart, and liver. Persistence data demonstrates that FT516-R cells are cleared within 28 days post-administration, which was confirmed also at 42 days post-administration, and with no evidence of permanent engraftment.
- Supporting the role of CD16 in enhancing ADCC, ligation of hnCD16 enhanced NK-cell activation as measured by calcium flux and phosphorylation of signaling cascades. Furthermore, the combination of FT516-R and antibodies, including rituximab, resulted in enhanced anti-tumor activity, as compared to antibody alone, FT516 alone, and peripheral blood NK cells alone.

2.4 Interleukin-2

IL-2 remains the only FDA approved drug that is capable of promoting NK cells activation and survival. There is also broad clinical experience and we have considerable clinical experience with the low doses of IL-2 proposed here. Although IL-2 may stimulate regulatory T cells (Treg), which can suppress NK cells, there are no other current options without exploring experimental agents (such as IL-15). Therefore, we believe that IL-2 is currently the best choice for this

first in human study while we explore the merits of IL-2 vs IL-15 and other cytokines in pre-clinical studies.

2.5 Lymphodepletion with Cyclophosphamide and Fludarabine

The importance of lymphodepleting chemotherapy has been well demonstrated in mouse experiments and was first proposed by the National Cancer Institute (NCI) in the context of melanoma specific CTL by Rosenberg and colleagues. (Dudley 2002) Lymphodepleting conditioning prior to adoptive transfer of lymphocytes is thought to promote persistence of adoptively transferred lymphocytes by creating "immunologic space" and providing a pool of homeostatic cytokines such as IL-15. The use of Cy/Flu-mediated lymphodepletion at the proposed doses and schedule is consistent with our previous human experience. (Miller 2005, Bachanova 2014)

2.6 Enoblituzumab

MGA271 (enoblituzumab, also known as RES242) is a humanized immunoglobulin (Ig) G1/kappa monoclonal antibody (mAb) that binds B7 homolog 3 (B7-H3), also referred to as CD276, a member of the B7 family of ligands that bind to receptors on lymphocytes and regulate immune responses. This agent is being developed by MacroGenics, Inc. B7-H3 is overexpressed on a number of tumor types and the primary mechanism of action for MGA271 is thought to be via antibody-dependent cellular cytotoxicity (ADCC). MGA271 comprises an engineered human IgG1 fragment crystallizable (Fc) domain that imparts increased affinity for the human activating Fc-gamma receptor (FcγR) IIIA (CD16A) and decreased affinity for the human inhibitory FcγRIIB (CD32B). (Enoblituzumab IB)

3 Study Rationale

3.1 Once Ovarian Cancer Recurs It Cannot be Cured

Ovarian cancer is the most lethal gynecologic malignancy. The estimated 5-year survival is 48% for all stages of ovarian cancer, and 29% for distant disease. Notably, 59% of women with ovarian cancer present with Stage III or IV disease, for which the rate of recurrence is 70 to 90% for Stage III and 90 to 95% for Stage IV. (SEERS) Women who recur cannot be cured with current therapies. Our long term objective is to exploit the innate immune system to treat ovarian cancer and substantially improve survival rates.

3.2 Rationale for Using Enoblituzumab

Clinical experience and trials have not identified an effective cancer antibody for ovarian cancer compared to others solid tumors responding to HER2 and EGFR targeted antibodies. Enoblituzumab is an Fc optimized humanized IgG1 monoclonal antibody that binds to B7-H3 (CD276). B7-H3 is widely expressed by a number of different tumor types, including a majority of ovarian cancers and ovarian cancer stroma (MacGregor 2019), and may play a key role in regulating the immune response. It has been reported that B7-H3 is expressed on at least 93% of ovarian tumors by immunohistochemical tissue microarray analysis on tumor specimens. (Zhang 2010) Interestingly, in the same study showed B7-H3 was expressed on 78% endothelium of tumor-associated vasculature from Stage III and IV patients but less so in early stage patients. This suggests that targeting B7-H3 in advanced ovarian cancer may dual target the tumors themselves and the tumor microenvironment in the advanced patients fitting eligibility for this trial.

3.3 Rationale for Using FT516 IP Dosing

Based on our published in vivo mouse data (Hermanson 2016) our current platform for NK cell therapy in ovarian cancer delivers NK cells IP as we have seen significant inhibition of ovarian cancer growth using this delivery. As ovarian cancer is an intraperitoneal disease, rarely metastasizing beyond the abdominal cavity, therapy delivered directly to the tumor site makes intuitive sense. Recent studies conducted by our collaborators defined the lymphocyte population within the peritoneal fluid (PF) of women with high-grade serous ovarian cancer (Figure 1). They found that women with ovarian cancer had a significantly lower percentage of NK cells in the PF compared to women with benign disease. Importantly, the ovarian cancer patients with lower number of NK cells had significantly lower survival.

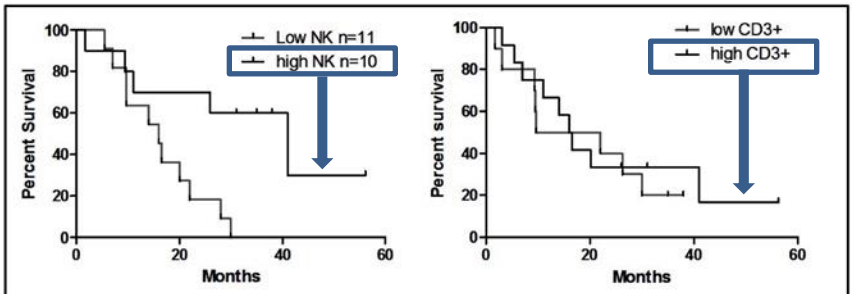


Figure 1: Kaplan Meier curve of the low NK group (<median) vs. patients with a high NK cell count in peritoneal fluid ((p<0.01). CD3 counts do not predict differences in survival. Data kindly provided by Dr. Harry Dolstra from the Radboud Institute for Molecular Life Sciences (RIMLS) at Radhoud University Medical Center.

Figure 1

This data provides the rationale for delivering the FT516 product directly within the peritoneal cavity. In addition, we recently completed a clinical trial where we

developed a 7-day culture process using a GSK3 inhibitor and IL-15 to manufacture modulated adaptive NK cells (FATE-NK100) from CMV^+ haploidentical donors for adoptive transfer. The phase I Apollo trial tested the maximum tolerated dose/maximum feasible dose (MTD/MFD) of FATE-NK100 administered intraperitoneally (IP) to treat platinum-sensitive or -resistant recurrent ovarian, fallopian tube, and primary peritoneal cancer. FATE-NK100 via IP port was tested using 3 dose cohorts ([DC]; 1×10^7 cells/kg; $>1 \times 10^7$ cells/kg to $\leq 3 \times 10^7$ cells/kg; or $>3 \times 10^7$ to $\leq 10 \times 10^7$ cells/kg) after lympho-conditioning with fludarabine 25 mg/m^2 IV and cyclophosphamide 300 mg/m^2 IV on days −6 and −5. After FATE-NK100 infusion on day 0, rhIL-2 at 6 million IU was given IP 3 times a week for 6 doses for in vivo NK activation. Nine patients were treated with no dose-limiting toxicities (DLTs). Retreatment based on clinical benefit was performed on 3 patients (33%), 2 following stable disease (DC 2) and 1 with partial remission (48% tumor reduction, DC 3). We found that the allogeneic product cells persist and have enhanced function compared to patient NK cells for up to 21 days, even after retreatment (our unpublished data).

We anticipate that the novel FT516 cell product will overcome the limitations of our current NK cell product, specifically by displaying better activity associated with longer survival after infusion into the peritoneal cavity. We have data showing that within the peritoneal microenvironment, the surface expression of CD16a on NK cells is significantly decreased presumed from ADAM17 mediated cleavage. This is one possible reason for decreased NK cell cytotoxicity in ovarian cancer that can be overcome with the use of the non-cleavable FT516 cell product. CD16a cell surface levels are tightly regulated by ADAM17 (Wu 2019, Romee 2013, Wang Y 2013, Jing 2015). We were the first to report that ADAM17 mediates this process by cleaving CD16a in a *cis* manner at a specific location proximal to the cell membrane. (Jing 2015) Though this is a normal process of immune homeostasis, it has been reported that NK cells in the tumor tissues of ovarian cancer patients

have significantly reduced levels of CD16a (Figure 2) and impaired anti-tumor effector functions (Lai 1996, Patankar 2005, Belisle 2007, Felices 2017).

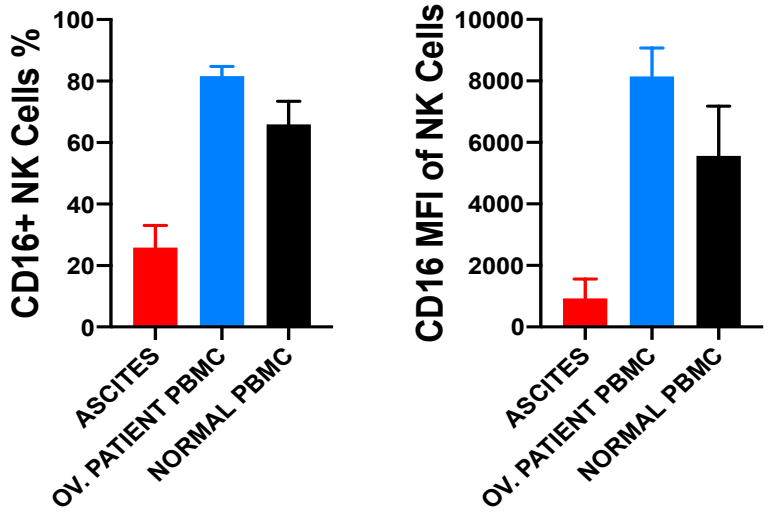


Figure 2. Percentage of CD16+ NK cells are significantly reduced in the peritoneal cavity compared to NK cells in the peripheral blood of ovarian cancer patients and in healthy patients.

4 Study Design

This is a single center Phase I clinical trial of FT516 administered intraperitoneally (IP) once a week for 3 consecutive weeks for the treatment of recurrent gynecologic cancers. As this combination is a first in human study and the first intraperitoneal infusion of FT516, the safety of FT516 is confirmed prior to adding enoblituzumab as an intravenous infusion approximately 1 week prior to the 1st dose of FT516. Each dose of FT516 is followed directly by an IP infusion of interleukin-2 (IL-2) to facilitate natural killer (NK) cell survival. A short course of outpatient lymphodepletion chemotherapy (CY/FLU) is given prior to the 1st dose of FT516.

FT516 is an off the shelf product comprised of allogeneic natural killer (NK) cells, expressing high-affinity non-cleavable CD16 (FT516). Enoblituzumab is a monoclonal antibody that targets B7-H3 which is highly expressed on ovarian cancer.

This study is conducted in two consecutive stages using the following treatment plan. In general:

- All patients are assessed for Dose Limiting Toxicity (DLT) as defined in Section 7.5. The study switches to Stage 2 (CRM) at any point in Stage 1 enrollment at the 1st DLT.
- A minimum of 28 days must separate each patient cohort (the last patient enrolled in the cohort completes the DLT assessment period)
- In multi-patient cohorts, a minimum of 14 days must separate the 1st and 2nd patient within a cohort.
- Stage 1 Step 2 is not used if a DLT occurs during Stage 1 Step 1.
- CRM is not used if no DLTs occur in Stage 1.

Cohort	Treatment Plan
1 (start)	IP FT516 monotherapy 9 x 10^7 cells/dose on Day 1, 8, and 15
2	IP FT516 monotherapy 3 x 10^8 cells/dose on Day 1, 8, and 15
3	IP FT516 monotherapy 9 x 10^8 cells/dose on Day 1, 8, and 15
4	Safe dose (MTD-1) from 1st 3 levels + IV enoblituzumab on Day -6
5	Highest dose (MTD) from 1st 3 levels + IV enoblituzumab on Day -6

Stage 1 Step 1 uses a fast-track design (1 patient per Cohort) with a minimum of 28 days between each patient until one of the following:

- The 1st occurrence of a pre-defined adverse event within 28 days of the cell infusion (defined as Grade 3 abdominal pain for > 48 hours despite standard analgesics or Grade 3 infusion related reaction) at which point the study moves to **Step 2** and the cohort size increases from 1 to 3 patients with 2 additional patients added to the current cohort. Escalation in Step 2 continues until the 1st DLT event at which point Stage 2 (CRM) is activated. If Cohort 5 is completed with 10 patients enrolled at the MTD without a DLT, Stage 2 (CRM) is not used.
- The 1st occurrence of a DLT at which point the study moves directly to Stage 2 (CRM) as detailed below and Step 2 is not used.
- Cohort 5 is completed without a pre-defined AE or a DLT – neither Step 2 nor Stage 2 is used if a total of 10 patients are enrolled in Cohort 5 to complete the study.

Stage 2 is initiated at the 1st DLT (as defined in Schema and in Section 7.5**)**. The study design changes to the continual reassessment method (CRM). Enrollment occurs in cohorts of three with a minimum of 14 days between the 1st and 2nd patient. Each new cohort of three patients are sequentially assigned to the most appropriate dose by the study statistician based on the updated toxicity probabilities once the 3rd patient in a cohort reaches Day 28 (end of DLT period). The MTD will be identified by

the minimum of the following criteria: (1) the total Stage 2 sample size of 25 is exhausted, (2) 10 consecutive patients are enrolled at the same dose plan or (3) the probability that the next 5 patients will be allocated to the same dose plan, based on the current estimate of the probability of toxicity, exceeds 90%.

For study endpoints, follow-up continues until disease progression and then for survival only for 1 year from the 1st dose of FT516.

After 1 year, follow-up will transfer to a separate long-term follow-up (LTFU) study to continue the FDA's required 15 year follow-up after treatment with a genetically modified cell therapy. A separate consent is required for the LTFU study.

5 Patient Selection

Study entry is open to all adult women regardless of race or ethnic background. While there will be every effort to seek out and include minority patients, the patient population is expected to be no different than that of other gynecological cancer studies at the University of Minnesota and other participating institutions.

5.1 Inclusion Criteria

5.1.1 Recurrent epithelial ovarian cancer, fallopian tube, or primary peritoneal cancer meeting one of the following minimal prior treatment requirement (no limit to the maximum number of prior treatments):

- Platinum Resistant: may receive FT516 as 2nd line (as 1st salvage therapy) with platinum resistant is defined as disease that has responded to initial chemotherapy but demonstrates recurrence within a relatively short period of time (< 6 months) following the completion of treatment.
- Platinum Sensitive: may receive FT516 as 3rd line therapy (as 2nd salvage therapy) with platinum sensitive is defined as the recurrence of active disease in a patient who has achieved a documented response to initial platinum-based treatment and has been off therapy for an extended period of time (≥ 6 months).

5.1.2 Measurable disease per modified Response Evaluation Criteria in Solid Tumors, v1.1 (iRECIST - refer to Appendix II) within the abdomen and pelvis assess within 42 days of the 1st FT516 infusion. Extra-peritoneal disease is permitted; however each lesion must be < 5 cm at the largest diameter.

5.1.3 At least 18 years of age

5.1.4 GOG Performance Status 0, 1, or 2 (refer to Appendix I)

5.1.5 Adequate organ function within 14 days of study registration (28 days for pulmonary and cardiac) defined as:

- Hematologic: platelets ≥ 75,000 x 10^9/L and hemoglobin ≥ 9 g/dL, unsupported by transfusions; absolute neutrophil count (ANC) ≥ 1000 x 10^9/L, unsupported by G-CSF or granulocytes
- Creatinine: Estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73m^2 per current institutional calculation formula
- Hepatic: AST and ALT ≤ 3 x upper limit of institutional normal
- Pulmonary Function: Oxygen saturation ≥ 90% on room air; PFT's required only if symptomatic or prior known impairment - must have pulmonary function >50% corrected DLCO and FEV1
- Cardiac Function: LVEF ≥ 40% by echocardiography, MUGA, or cardiac MRI; no clinically significant cardiovascular disease including any of the following: stroke or myocardial infarction within 6 months prior to first study treatment; unstable angina or congestive heart failure of New York Heart Association Grade 2 or higher (Appendix I)

5.1.6 Agrees to the placement of an intraperitoneal catheter before the 1st dose of study directed drug (chemotherapy or enoblituzumab - Cohort 4 and 5) and remains in place through Day 36 or longer if retreatment is planned

5.1.7 Agrees to undergo a tumor biopsy if feasible at the time the catheter is placed and removed – Accessible tumor for biopsy is not required for eligibility.

5.1.8 Washout period of at least 14 days after any standard of care tumor directed therapy prior to the first dose of investigational product (FT516 for Levels 1-3 or enoblituzumab for Levels 4-5)

5.1.9 If history of brain metastases must be stable for at least 3 months after treatment – A brain CT scan or MRI is only be required in subjects with known brain metastases at the time of enrollment or in subjects with clinical signs or symptoms suggestive of brain metastases

5.1.10 Must agree to and sign the consent for the companion Long-Term Follow-Up study (CPRC #2020LS072) to fulfill the FDA required 15 years of follow-up for a genetically modified cell product

5.1.11 Voluntary written consent prior to the performance of any research related procedures

5.2 Exclusion Criteria

5.2.1 Pregnant or breastfeeding or planning on becoming pregnant in the next 6 months. Woman of childbearing potential who still have a uterus and ovaries, must agree to use at effective contraception and must have a negative pregnancy test within 14 days of study enrollment.

5.2.2 Any known condition that requires systemic immunosuppressive therapy (> 5mg prednisone daily or equivalent) during the FT516 dosing period (3 days before the 1st dose through 14 days after the last dose) – topical and inhaled steroids are permitted.

5.2.3 Active autoimmune disease requiring systemic immunosuppressive therapy

5.2.4 History of severe asthma and currently on chronic systemic medications (mild asthma requiring inhaled steroids only is eligible)

5.2.5 Uncontrolled bacterial, fungal or viral infections with progression of clinical symptoms despite therapy

5.2.6 Receipt of any investigational agent within 28 days prior to the first dose of investigational product (FT516 for Levels 1-3 or enoblituzumab for Levels 4-5)

5.2.7 Live vaccine <6 weeks prior to start of lympho-conditioning

5.2.8 Known allergy to the following FT516 components: albumin (human) or DMSO

5.2.9 Any history of prior enoblituzumab administration

5.2.10 Known history of HIV positivity or active hepatitis C or B - chronic asymptomatic viral hepatitis is allowed

5.2.11 Presence of any medical or social issues that are likely to interfere with study conduct or may cause increased risk to patient

6 Patient Screening and Study Enrollment

6.1 Registration with the Masonic Cancer Center Clinical Trials Office

Any patient who is consented is to be entered in OnCore by the site Study Coordinator or designee.

If a patient is consented but is not enrolled in the study treatment (i.e. is found to be ineligible based on pre-transplant inclusion/exclusion criteria), the patient's record is updated in OnCore as a screen failure and reason for exclusion recorded.

6.2 Patient Enrollment and Cohort Assignment

To be eligible for study treatment, the patient must sign the treatment consent and meet each inclusion criteria and none of the exclusion criteria on the eligibility checklist based on an eligibility assessment documented in the patient's medical record.

Up to 5 patient cohorts of FT-516 (with enoblituzumab in Cohort 4 and 5) are tested in this study. The patient is assigned to the currently enrolling cohort once eligibility is confirmed.

6.3 Patient Re-Treatment with FT516

If a patient achieves an initial response (iCR, iPR, iSD) at the Day 36 reassessment, she may be eligible to repeat her assigned study treatment (CY/FLU lymphodepletion and 3 weekly doses of FT516/IL-2). If the patient initially was assigned to Cohort 4 or 5, it must be coordinated with the 3rd dose of enoblituzumab.

If a patient achieves iUPD at the Day 36 reassessment, they will be given the option of keeping their IP catheter in place, re-scanning in 4 weeks, and if they have attained iSD, iPR, or iCR at that time, they may be eligible to repeat their assigned study treatment. If a patient achieves iUPD or iCPD at the re-scan, they will not be eligible for retreatment.

A patient may be considered for retreatment if the following criteria is met (in addition to at least stable disease based on iRECIST):

- The patient did not experience unacceptable toxicity (experienced DLT equivalent toxicity) or toxicity that caused a dose to be skipped during the first course of FT516.
- All adverse events have resolved to ≤ Grade 1 or baseline, whichever is higher.

- No decline in GOG performance status from baseline.
- The patient signs a re-treatment consent.

After confirmation of eligibility and signing of a re-treatment consent, the patient will be registered to the re-treatment study (2020LS001R) in OnCore releasing a new study calendar. Retreatment will have no effect on the primary and secondary analysis; however, adverse events are documented and reported per Section 10. Study endpoints will remain at 6 months and 12 months from 1st FT516 infusion.

6.4 Patients Who Do Not Begin Study Treatment

If a patient is registered to the study and is later found not able to begin study treatment (beginning with the 1st dose FT516 for Cohorts1-3 or 1st dose of enoblituzumab for Cohorts 4 and 5), the patient will be removed from the study and treated at the physician's discretion. The study staff will update OnCore with the patient's non-treatment status. The reason for removal from study must be clearly indicated in OnCore. The patient will be replaced to complete enrollment.

7 Treatment Plan

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care drug therapy (i.e. acetaminophen, diphenhydramine, antimicrobials, etc.).

There is some flexibility in the timing of the treatment as long as the ordering of the treatment is maintained and a minimum of 48 hours separates the last dose of fludarabine and the 1st FT516 infusion.

7.1 Peritoneal Catheter Placement (Day – 6 or earlier)

Successful placement of the IP catheter is required before proceeding with the lymphodepleting chemotherapy. If an IP catheter cannot be placed, the patient will be taken off study and replaced.

The peritoneal site where the catheter is to penetrate the peritoneum is identified under direct vision by Interventional Radiology. It is important to enter the peritoneal cavity under direct vision because a previous staging laparotomy for ovarian cancer is likely to have resulted in adhesion formation, particularly between bowel and anterior abdominal wall. The catheter insertion site through the peritoneum must be separate from other incisions.

There is a risk for spread of cancer cells that are located within the peritoneum to surrounding tissues such as the abdominal wall. This risk is associated with the

penetration of the abdominal cavity during the IP catheter placement as it exposes new surfaces for the cancer cells to attach. Implantation of malignant cells within surgical incisions and along biopsy needle and drainage catheter tracts is a recognized mechanism for the dissemination of cancer. In one review published of 255 patients with epithelial ovarian carcinoma, Dauplat et al. (Dauplat, et al. 1987) included nine cases (3.5%) with skin metastases, one of which occurred at the site of a catheter used for intraperitoneal chemotherapy. Kohler et al. (Kohler, et al. 1991) described two patients who were treated with intraperitoneal administration and subsequently developed abdominal wall metastases. In one case the lesions were at the site of previous surgical incisions. In the other, metastasis occurred at the former site of the peritoneal access catheter which had been removed after therapy 2.5 years earlier. Implantation metastases is a potential hazard of all invasive procedures in the case of malignancy. Although thought to be a rare event, placement of a peritoneal access catheter in ovarian cancer may be a risk for seeding tumor cells.

The baseline research related peritoneal cell collection (ascites or intraperitoneal washing) and a tumor biopsy, if feasible will be done at placement (and removal). Refer to Section 9.2.1 and Section 9.2.2.

The catheter will remain in place until at least Day 36 for peritoneal IP cell collection and biopsy or longer, if the patient agrees to and is eligible for a 2nd treatment course.

7.2 Enoblituzumab (Day -6 then Day 22 and every 21 Days thereafter) - Cohort 4 and 5 only

Enoblituzumab 15 mg/kg is given as an intravenous infusion over 120 minutes (±15 minutes) using a commercially available pump. A window of ±2 days is permitted. The dose of enoblituzumab initially is calculated based on the patient's actual weight. Treatment is administered in the outpatient clinic.

Obtain vital signs (temperature, blood pressure, respiration and pulse) upon arrival, within 15 minutes of starting the enoblituzumab, every 15 minutes (± 5 minutes for the first hour of the infusion, every 30 minutes (±5 minutes) thereafter until infusion end, upon discontinuing infusion, and before the patient is discharged from the clinic.

The 1st dose of enoblituzumab is given Day -6 (±2 days) and at least 1 day before the start of CY/FLU preparative regimen.

Subsequent doses of enoblituzumab are given every 21 days (±2 days) starting on Day 22 using the 1st dose of FT516 as Day 1. Treatment may continue until disease progression or unacceptable side effects.

If a patient is eligible for re-treatment per Section 6.3, the 3rd dose of enoblituzumab (around Day 43) serves as the anchor for CY/FLU and FT-516/IL-2 administration.

7.2.1 Pre-Medications and Prophylaxis Guidelines

Required prior to the first infusion of enoblituzumab:

- Acetaminophen 650-1000 mg orally (PO) or ibuprofen 400 mg PO, or equivalent
- Diphenhydramine 50 mg PO or IV or equivalent H1 antagonist
- Ranitidine 300 mg PO or IV or equivalent H2 antagonist
- Dexamethasone 10-20 mg IV.

No pre-infusion prophylactic steroids administration is required for subsequent infusions. Non-steroidal pre-medications may be administered prior to the subsequent infusion, if warranted.

Antiemetic therapy including but not limited to neurokinin 1 (NK1) receptor antagonists, serotonin (5HT3) receptor antagonists, benzodiazepines, antihistamines, cannabinoids, cholinergic antagonists and dopamine receptor antagonists may be administered according to good clinical practice and local guidelines.

If the patient experiences a ≥ Grade 2 infusion related reaction with the first infusion refer to the recommended pre-medications found in the Grade 2 Infusion Related Reaction in Section 7.2.2. At Day 22 and beyond steroid use is permitted as a pre-med in the setting of a recurrent Grade 2 or Grade 3 infusion related reaction.

7.2.2 Management of Observed Infusion Related Reactions

Grade 3 or greater infusion related reactions are reportable as a DLT (if within 28 days of the 1st FT516 infusion), AESIs and/or SAEs, as applicable per Section 9.2.

The following are treatment guidelines (which may be modified as needed by the Investigator according to the best practices of medicine) for infusion reactions:

Grade 1 Infusion Related Reaction:

- Slow the infusion rate by 50%.
- Monitor the patient for worsening of condition.
- Continue rate at 50% reduction and increase dose rate to the original rate by doubling the infusion rate after 30 minutes, as tolerated to the initial rate.

Consideration can be given to beginning subsequent infusions at 50% rate and increasing as tolerated.

- The following prophylactic pre-infusion medications are recommended prior to future infusions of study treatment for patients who experience Grade 1 infusion reactions:
  - Diphenhydramine 25 to 50 mg (or equivalent) PO/IV.
  - Acetaminophen 650 mg PO and/or ibuprofen 400 mg PO, or equivalent, at least 30 minutes before additional study drug administrations.
  - Ranitidine 300 mg PO or 50 mg IV or equivalent H2 antagonist before additional study drug administrations.

Grade 2 Infusion Related Reaction:

- Stop the infusion.
  - Administer diphenhydramine hydrochloride 25 to 50 mg IV.
  - Acetaminophen 650 mg PO or ibuprofen 400 mg PO, or equivalent, for fever.
  - Oxygen and bronchodilators for mild bronchospasm.
- Resume the infusion at 50% of the prior rate once the infusion reaction has resolved or decreased to Grade 1. The rate may then be escalated to the original rate after 30 minutes, as tolerated. Consideration can be given to beginning all subsequent infusions at 50% rate and increasing as tolerated.
- Monitor for worsening condition. If symptoms recur, discontinue the infusion; no further study drug will be administered at that visit.
- Prophylactic pre-infusion medications should be given prior to subsequent infusions of study treatment.
  - Patients who experience Grade 2 infusion reaction, for subsequent doses of study treatment, pre-medicate with diphenhydramine hydrochloride 25 to 50 mg IV/PO and acetaminophen 650 mg PO and/or ibuprofen 400 mg PO (or equivalent) at least 30 minutes before additional study drug administrations.
  - For patients with Grade 2 infusion reactions, despite premedication with diphenhydramine and acetaminophen and/or ibuprofen (or equivalent), corticosteroids (10 to 20 mg dexamethasone IV or equivalent) may be added to the premedication regimen for the next dosing of study treatment. Reduce corticosteroid dosing by 50% for the subsequent dose and hold thereafter, if there are no reactions.

Grade 3 Infusion Related Reaction:

- STOP THE INFUSION AND DISCONNECT THE INFUSION TUBING FROM THE PATIENT.

- TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING – ASPIRATE RESIDUAL DRUG FROM THE PORT LUMEN.
- Administer diphenhydramine hydrochloride 25 to 50 mg IV, dexamethasone 20 mg IV (or equivalent), and other medications/treatment as medically indicated. Higher doses of corticosteroids (e.g., methylprednisolone 2 to 4 mg/kg IV or the equivalent) may also be considered for acute management.
- Consider administering, tocilizumab (an IL6 receptor antagonist) 4 mg/kg IV.
- IV fluids, supplemental oxygen, H2 blockers such as ranitidine and bronchodilators should be considered, as appropriate.
- If the Grade 3 infusion reaction occurs with any study treatment, it will be discontinued for that day.
    - If symptoms have resolved to baseline within 12 hours, study treatment may be infused at the next scheduled dose, with a 50% reduction of infusion rate. In addition, patients should be pre-medicated for this re-challenge and for any subsequent doses of study treatment with the following: diphenhydramine hydrochloride 25 to 50 mg IV, acetaminophen 650 mg PO and/or ibuprofen 400 mg PO (or equivalent); corticosteroids should be considered as well (dexamethasone 10 to 20 mg IV or equivalent). Reduce corticosteroid dosing by 50% for the subsequent dose and hold thereafter, if there are no reactions.
    - Patients who have a Grade 3 infusion reaction that does not resolve within 12 hours despite medical management should be discontinued from study treatment.
- Patients who experience a second Grade 3 infusion reaction at the time of re challenge of study treatment (the same study treatment for which the first Grade 3 infusion reaction was seen) will permanently discontinue study treatment.

Grade 4 Infusion Related Reaction:

- STOP THE INFUSION AND DISCONNECT THE INFUSION TUBING FROM THE PATIENT.
- TO AVOID EXACERBATION OF INFUSION REACTION OR CRS: DO NOT FLUSH THE TUBING. ASPIRATE RESIDUAL DRUG FROM THE PORT LUMEN
- Administer diphenhydramine hydrochloride 50 mg IV, dexamethasone 20 mg IV (or higher doses of steroids, e.g., methylprednisolone 2 to 4 mg/kg IV or the equivalent, as considered appropriate).
- Consider administering, tocilizumab (an IL6 receptor antagonist) 4 mg/kg IV.
- Give epinephrine or bronchodilators as indicated.

- Support ventilation and blood pressure as indicated.
- Patients who have a Grade 4 infusion reaction will permanently discontinue study treatment.

7.3 Preparative Regimen (Day -5 and Day -4)

A lymphodepleting regimen of fludarabine and cyclophosphamide is given in the outpatient clinic on two consecutive days. If there is unresolved toxicity associated with the enoblituzumab, treatment may be delayed; however, the second day of CY/FLU must be no later than Day -2 to ensure a minimum of a 48 hour wash-out period prior to the FT516 cell infusion.

The administration of the preparative regimen will follow the institutional dosing guidelines. Dose and/or schedule adjustments consistent with the standard of care may be made on an individual patient basis as needed for safety.

Fludarabine 25 mg/m^2 is administered as a 1 hour intravenous (IV) infusion per institutional guidelines once a day on 2 consecutive days.

Cyclophosphamide 300 mg/m^2 is administered as a 2 hour intravenous infusion per institutional guidelines once a day on the same 2 days fludarabine is given.

Cyclophosphamide dosing is calculated based on ABW (Actual Body Weight) unless ABW is >150% of the IBW (Ideal Body Weight).
Adjusted body weight = IBW + 0.5(ABW-IBW).

Cyclophosphamide associated hydration will be given according to recommended institutional standards.

7.4 FT516 and IL-2 (Day 1, Day 8, and Day 15)

A minimum of 48 hours must separate the last dose of fludarabine and the 1st dose of FT516. The 1st dose of FT516 may be delayed for up to 7 days after the last dose of fludarabine if the study physician feels a delay is in the best interest of the patient. The 1st dose of FT516 equals Day 1.

A ± 1 day window is permitted for subsequent infusions to accommodate scheduling issues.

Decision to Treat on Day of Planned FT516: Three weekly doses of FT516 are planned; however, if on the day of treatment, the study physician feels it is not in the best interest of the patient to receive treatment, the FT516/IL-2 may be:

- Delayed for up to 48 hours adjusting future time points to maintain timing OR
- Skipped and the patient scheduled for the next planned visit. Skipped dose(s) are not made up.

Pre-medications: Prior to the administration of FT516 and IL-2 and 4 hours later, patients should be given acetaminophen 650 mg orally and diphenhydramine 25 mg orally. Corticosteroids should not be used as pre-medication for FT516.

If a large amount of ascites is present it will be drained prior to instillation of the FT516 and IL-2. Refer to Section 9.2.1 regarding submitting ascites, if present, for research related testing.

FT516 Infusion guidelines: FT516 is provided in one or more cryopreserved bags depending on the patient's assigned FT516 cell dose (Level 1: 9 x 10^7 cells, Level 2: 3 x 10^8 cells, or Level 3: 9 x 10^8 cells). Thawing of the bag(s) is according to the FT516 Storage, Handling, and Administration Guidance.

FT516 must be administered using an IV administration set without an in line filter (filter is not required for IP administration). FT516 is administered as an IP infusion via gravity.

Each bag and tubing then will be flushed with 50 cc of room temperature normal saline. Prior to the IL-2, flush the line a final time with 50 cc of D5W at room temperature.
IL-2 administration: IL-2 at 6 MIU (3 MIU/m^2 if patient weight is <45 kg) is administered IP in 50 cc of room temperature D5W via peritoneal catheter directly after the FT-516 infusion is completed. Immediately following the IL-2, an additional 100 cc D5W flush is infused into the peritoneal cavity through the peritoneal catheter as rapidly as possible.

After the 100 cc D5W flush, the patient is asked to change position at 15-minute intervals for two hours to ensure adequate intra-abdominal distribution. No attempt will be made to retrieve the infusate.

Vital Signs include oral temperature, systolic and diastolic blood pressure, heart rate, respiratory, and pulse oximetry. On days of FT516 administration, collect as follows: within 15 minutes prior to the infusion of FT516, at 10 (±5) minutes during infusion, and every 15 (±5) minutes for 1 hour following the end of infusion of the last administered bag of FT516

Monitoring for Acute Adverse Events of FT516:
In our previous study of intraperitoneal (IP) Fate NK100 and IL-2 minimal toxicity was seen and primarily was related to abdominal pain/cramping.

Patients are monitored for infusion related reactions. If an infusion reaction of any grade occurs do not give any remaining bags of cells (if relevant). Refer to Table 1 for management guidelines based on infusion related reaction CTCAE v5 grading.

Dose modification guidelines for IL-2 related toxicity:
Grade 3 adverse event: if the toxicity resolves to grade 2 or better within 48 hours, the IL-2 can be resumed at a reduced dose (4 million units or 2 million units/m^2 if < 45 kg). If after a dose reduction the same toxicity persists, worsens, or recurs, the IL-2 must be permanently discontinued.
Grade 4 adverse event: permanently discontinue IL-2

7.5 Monitoring for Dose Limiting Toxicities

All patients are monitored for dose limiting toxicities for 28 days after the 1st FT516 infusion.

In addition, during the initial fast-track dose escalation (Stage 1, Step 1), patients also are monitored under a separate set of criteria for abdominal pain and infusion related reaction for 28 days post infusion as defined in the Schema and Study Design. The 1st occurrence of an event triggers a switch from enrolling 1 patient per cohort to 3 patients per level (Stage 1, Step 2) and monitoring for dose limiting toxicity. If in Stage 1, Step 1, a patient experiences a DLT, enrollment moves directly to Stage 2 using CRM.

DLT is defined as any treatment emergent toxicity at least possibly related to the study treatment meeting one of the following criteria based on CTCAE v5 within 28 days (14 days for ascites) of the 1st FT516 infusion (for Cohort 4 and 5, DLT assessment starts with enoblituzumab and continues for 28 days after 1st FT516):

- Grade 3 organ toxicity (pulmonary, hepatic, renal, or neurologic) not pre-existing and lasting more than 72 hours
- Any non-hematologic Grade 4 or 5 toxicity
- Neutrophil count decreased ≥ Grade 4 that persists at Day 28 despite use of growth factor support
- Grade 3 abdominal pain lasting more than 4 consecutive days and not controlled by standard analgesics

- Grade 3 or greater ascites within 14 days after FT516 administration in patients who had no ascites or Grade 1 ascites at enrollment and is not attributable to disease progression

7.6 Supportive Care

Throughout the study, the investigator may prescribe any concomitant medications or treatment deemed necessary to provide adequate supportive care. Supportive care may include antibiotics, anti-fungals, analgesics, transfusions, growth factors.

7.7 General Concomitant Medication Guidelines

Because they may inhibit NK cell function, systemic corticosteroids should be avoided 3 days before the 1st dose of FT516 through 14 days after the last FT516 infusion unless absolutely required. Dexamethasone as premedication for cyclophosphamide and fludarabine may be administered per the USPI.

Corticosteroids should not be used as pre-medication for FT516.

Beta-blockers and other anti-hypertensives may potentiate hypotension associated with IL-2, especially if the patient is not well hydrated.

7.8 Duration of FT516 / IL-2 Treatment and Possible Retreatment

Treatment with FT516 and IL-2 consists of a single treatment course of 3 weekly doses on Day 1, Day 8, and Day 15 (with 2 doses of enoblituzumab if assigned to Cohort 4 or 5).

If a patient achieves an initial response (iCR, iPR, iSD) at the 1st disease re-assessment around Day 36, she may be eligible to repeat the treatment at her same FT516 cohort provided the criteria in Section 6.3 is met. If a patient achieves iUPD at the Day 36 reassessment, they will be given the option of keeping their IP catheter in place, re-scanning in 4 weeks, and if they have attained iSD, iPR, or iCR at that time, they may be eligible to repeat their assigned study treatment. If a patient achieves iUPD or iCPD at the re-scan, they will not be eligible for retreatment.

Retreatment will have no effect on the primary and secondary analysis; however, adverse events are documented and reported per Section 10. Study endpoints will remain 6 months and 12 months from 1st FT516 infusion.

7.9 Duration of Enoblituzumab (COHORT 4 or 5 ONLY)

Enoblituzumab may continue every 3 weeks from Day 22 until disease progression and/or unacceptable toxicity or patient refusal. If a patient is eligible for retreatment with FT516/IL-2 this would occur in association with the 3rd dose of enoblituzumab.

7.10 Duration of Study Participation

Patients will be followed for disease response until disease progression/relapse and then survival only for 12 months from the 1st FT516 infusion unless:

- consent is withdrawn
- patient did not receive FT516 – if a patient is not evaluable, follow only until the resolution or stabilization of treatment related toxicity
- new anti-cancer treatment is started (follow for survival only)
- patient is discharged to hospice (terminal) care

If applicable, enoblituzumab may continue every 3 weeks beyond 12 months in the absence of disease progression and unacceptable toxicity.

Any patient receiving at least 1 dose of FT516 must be followed for up to 15 years for late effects and survival. After the visit at 12 months from the FT516 the patient transfers to the long-term follow-up protocol (CPRC# 2020LS072) separate consent required at enrollment into this treatment study) and continue to be followed for safety, anti-tumor activity, and survival for up to 15 years from the 1st FT516 infusion.

8 Management of Selected Expected Toxicities Associated with Enoblituzumab and/or FT516/IL-2 Administration

See Appendix III for expected toxicities of the lymphodepleting regimen and IL-2.

8.1 Known Infusion Related Reaction (IRR) to Enoblituzumab (Cohort 4 and 5)

Refer to Section 7.2.2 for the management of enoblituzumab related infusion reaction.

To date, the most important safety risk that has been identified with enoblituzumab is infusion-related reactions (IRR), including reactions known as cytokine release syndrome (CRS) due to the release of small proteins (cytokines) from the cells. IRRs are generally temporary effects due to a drug that may occur during or shortly after infusion of the drug.

Signs and symptoms of an infusion-related reaction may include fever, chills, nausea, vomiting, headache, muscle stiffness, rash, itching, low blood pressure, and difficulty breathing. IRRs can be life threatening and, in rare cases, may cause death. For all adult studies, IRRs (including CRS) have occurred in a total of 47.6 % (between 4-5 out of 10) participants receiving treatment with enoblituzumab.

Most of the IRRs observed in patients receiving enoblituzumab have been mild to moderate in severity. However, five patients receiving enoblituzumab alone and twelve patients on enoblituzumab in combination with checkpoint blockade have had severe IRRs. Two participants experienced IRR/CRS that were considered life-threatening. These patients, some of whom were hospitalized for these reactions, recovered after receiving treatment with steroids, antihistamines and intravenous fluids. Three patients discontinued from the treatment after experiencing an IRR. Based on these known IRR, a management plan has been developed by MacroGenics, the drug manufacturer.

8.2 Infusion Related Reaction Secondary to the Infusion of FT516

In our previous study of intraperitoneal (IP infusion) Fate NK100 and IL-2 minimal toxicity was seen and primarily was related to abdominal pain/cramping.

Patients are monitored for acute infusion related reactions associated with intravenous infusions including rigors and chills, rash, urticaria, hypotension, dyspnea, and angioedema during and after IP infusion.

Patients should be monitored for the occurrence of hypotension, dyspnea, and angioedema during and immediately after the infusion. Appropriate medical care should be instituted as per the respective current local prescribing information or standard institutional practices. Because they may inhibit NK cell function, systemic corticosteroids should be avoided unless absolutely required for management of acute allergic/infusion reactions, as determined by the Investigator.

Refer to Table 1 for management guidelines based on infusion related reaction CTCAE v5 grading.

Table 1: Recommended Guidelines for the Management of Acute Allergic/Infusion Reaction with FT516 Administration	
Grade	**Management**
Any Grade	• Interrupt FT516 administration. • Manage symptoms, e.g., with antihistamines, antipyretics and analgesics, according to standard institutional practice standards
Grade ≤3	• Resume FT516 administration only upon complete resolution of the infusion-related reaction and at the discretion of the Investigator. Given that FT516 administration may involve single or multiple bags depending on the total planned dose and accounting for the stability of FT516 post-thaw, FT516 administration may continue following resolution of Grade ≤3 infusion-related reactions as follows: If single-bag FT516 dosing: – No additional FT516 may be administered. – The volume of FT516 administered prior to the infusion-related reaction must be documented; retain any remaining product and contact the Sponsor for further instruction. – Additional bags may not be administered to make up for FT516 that was not administered from the bag during which the infusion-related reaction occurred. If multiple-bag FT516 dosing: - The volume of FT516 administered from the bag during which the infusion-related reaction occurred must be documented; retain any remaining product from the bag and contact the Sponsor for further instruction. - If dosing with additional FT516 bags was planned, they may be thawed and administered. - Additional bags beyond what was originally planned may not be administered to make up for FT516 that was not administered from the bag during which the infusion-related reaction occurred.
Grade	**Management**
Grade 4	• Stop FT516 administration. Do not restart. The volume of FT516 administered prior to the infusion-related reaction must be documented; retain any remaining product and contact the Sponsor for further instructions.

8.3 DMSO-Related Risks

FT516 is formulated in DMSO to enable cryopreservation. Intravenous DMSO side effects and symptoms are generally associated with histamine release and include coughing, flushing, rash, chest tightness and wheezing, nausea and vomiting, and cardiovascular instability. However, due to the small cell bag volume (~28 ml) and intraperitoneal infusion, a reaction to DMSO is not expected.

8.4 Infection

FT516 is cell therapy of human origin. During processing, the cells are in contact with reagents of animal origin, and FT516 has a final formulation which contains albumin (human). As with any product of human and/or animal origin, transmission of infectious disease and/or disease agents by known or unknown agents may

occur. FT516 has been extensively tested to minimize the potential risk of disease transmission. However, these measures do not completely eliminate the risk. For some infectious agents, there are no routine tests to predict or prevent disease transmission.

8.5 IL-2

IL-2 when given subcutaneously is associated with constitutional symptoms (flu-like symptoms) which may be dose limiting. IL-2 related toxicities associated with subcutaneous IL-2 are found in Appendix III.

In this study IL-2 is administered directly after FT516. Prior to administration of FT516 and 4 hours later, it is recommend patients are given acetaminophen 650 mg orally and diphenhydramine 25 mg orally to reduce or avoid expected toxicities of both FT516 and IL-2.

In a study of women with platinum resistant or refractory ovarian cancer the administration of single agent IL-2 weekly at dose higher than used in this study was generally well tolerated. Grade 1 and 2 constitutional symptoms (flu-like symptoms, GI and neurological) were most common, but controlled with medication and not requiring dose reduction (Vlad).

8.6 Cytokine Release Syndrome (CRS) or CRS-Like Symptoms

While CRS is a clearly defined syndrome in T cell therapy, it is not known to occur to the full extent in NK cell therapies. However, we have seen immune activation syndromes with other IL-15 products that include fever, CNS toxicities, and rash but without T cell mediated vascular collapse.
If CRS is suspected, CRP and ferritin levels should be assessed locally, and a serum sample collected for an IL-6 level (if testing available at the treating institution).

If indicated by the presence of medically significant symptoms and/or high IL-6 levels or any symptoms requiring intervention, steroids are the first line of treatment.

To consistently characterize its severity, CRS must be graded as outlined in the ASTCT CRS consensus grading system provided in Table 4 (Section 10). Because the signs and symptoms of CRS are not unique to CRS, other causes of fever, hypotension and/or hypoxia must be excluded. Notably, bacteremia and other severe infections have been reported concurrent with and even mistaken for CRS (Lee et al. 2019).

If CRS occurs (e.g. a differential diagnosis is recorded in the institutional medical record), CRP and ferritin levels should be done three times weekly until the resolution of CRS per Section 8.1. In addition, a serum sample should be collected for an IL-6 level at the time of any change (increase or decrease) in the CRS grade. Because patients may be outpatients any missed collection time points will not be a protocol deviations.

Management of CRS should follow the recommended management algorithm provided in Table 2 (Neelapu 2018) and/or institutional practice.

Table 2: Recommendations for the Management of Cytokine Release Syndrome

Grade	Sign/Symptom	Management
Grade 1	Fever or organ toxicity	• Acetaminophen and hypothermia blanket for the treatment of fever • Ibuprofen can be used as second treatment option for fever, if not contraindicated • Assess for infection using blood and urine cultures, and chest radiography • Empiric broad-spectrum antibiotics and filgrastim if neutropenic • Maintenance IV fluids for hydration • Symptomatic management of constitutional symptoms and organ toxicities • Consider tocilizumab 8 mg/kg [a] IV or siltuximab 11 mg/kg IV for persistent (lasting ≥3 days) and refractory fever
Grade 2	Hypotension	• IV fluid bolus of 500–1,000 mL of normal saline • Can give a second IV fluid bolus if systolic blood pressure remains <90 mmHg • Tocilizumab 8 mg/kg [a] IV or siltuximab 11 mg/kg IV for the treatment of hypotension that is refractory to fluid boluses; up to 3 additional doses of tocilizumab may be administered, and the interval between consecutive doses should be at least 8 hours. • If hypotension persists after two fluid boluses and anti-IL-6 therapy, start vasopressors, consider transfer to ICU, obtain echocardiogram, and initiate other methods of hemodynamic monitoring • In subjects at high-risk [b] or if hypotension persists after 1–2 doses of anti-IL-6 therapy, dexamethasone can be used at 10 mg IV every 6 hours • Manage fever and constitutional symptoms as in Grade 1
	Hypoxia	• Supplemental oxygen • Tocilizumab or siltuximab ± corticosteroids and supportive care, as recommended for the management of hypotension
	Organ toxicity	• Symptomatic management of organ toxicities, as per standard guidelines • Tocilizumab or siltuximab ± corticosteroids and supportive care, as indicated for hypotension

Table 2: Recommendations for the Management of Cytokine Release Syndrome		
Grade	Sign/Symptom	Management
Grade 3	Hypotension	• IV fluid boluses as needed, as recommended for the treatment of Grade 2 CRS • Tocilizumab and siltuximab as recommended for Grade 2 CRS, if not administered previously • Vasopressors as needed • Transfer to ICU, obtain echocardiogram, and perform hemodynamic monitoring as in the management of Grade 2 CRS • Dexamethasone 10 mg IV every 6 hours; if refractory, increase to 20 mg IV every 6 hours • Manage fever and constitutional symptoms as indicated for Grade 1 CRS
	Hypoxia	• Supplemental oxygen including high-flow oxygen delivery and non-invasive positive pressure ventilation • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above
	Organ toxicity	• Symptomatic management of organ toxicities as per standard guidelines • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above
Grade 4	Hypotension	• IV fluids, anti-IL-6 therapy, vasopressors, and hemodynamic monitoring as defined for the management of Grade 3 CRS • Methylprednisolone 1 g/day IV • Manage fever and constitutional symptoms as in Grade 1 CRS
	Hypoxia	• Mechanical ventilation • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above
	Organ toxicity	• Symptomatic management of organ toxicities as per standard guidelines • Tocilizumab or siltuximab plus corticosteroids and supportive care, as described above

CRS, cytokine release syndrome; ICU, intensive care unit; IV, intravenous.

NOTE: All medication doses indicated are for adults.

[a] Maximum amount of tocilizumab per dose is 800 mg.

[b] High-risk subjects include those with bulky disease and those with comorbidities.

Reference: Neelapu 2018, Actemra USPI

8.7 Tumor Lysis Syndrome

Tumor lysis syndrome (TLS) is a possible fatal risk associated with anti-tumor therapy. TLS symptoms include nausea, vomiting, diarrhea, muscle cramps or twitches, weakness, numbness or tingling, fatigue, decreased urination, irregular heart rate, restlessness, irritability, delirium, hallucinations, and seizures. TLS is comprised of abnormal lab changes that include hyperuricemia, hyperkalemia, hyperphosphatemia, and hypocalcemia. TLS has been reported to occur within 7 days following chemotherapy across various solid tumor settings, with ten published reports of TLS cases in patients with gynecological cancer (Mirrakhimov 2014).

One case of fatal metabolic syndrome compatible with TLS was reported following NK cell therapy in a patient with ovarian cancer (Geller 2011) 5 days after receiving CY. Prophylaxis for and management of TLS should be done in accordance with standard institutional practice.

8.8 Neurotoxicity

While CNS toxicity is a clearly defined syndrome associated with T-cell-based therapies or with high-dose IL-2, it is rare and generally not believed to be a toxicity associated with NK cell therapies, including those given with IL-2 at these doses. Neurotoxicity was reported in one trial of adoptively transferred NK cells given with SC IL-15 but the mechanism of the toxicity was not well defined (Cooley et al. 2019). Nervous system toxicities following CD19 CAR-T therapy is characterized by encephalopathy, confusion, delirium, aphasia, obtundation, and seizures (Kymriah USPI 2018; Yescarta USPI 2019). Cases of cerebral edema have also been reported (Brudno 2016).

To consistently characterize its severity, neurotoxicity must be graded using the ASTCT guidelines for grading ICANS provided in Section 10 based on the Immune Effector Cell-Associated Encephalopathy (ICE) score and Table 3. Per Section 9 an assessment is done prior to each FT516/IL-2 infusion, 2 to 4 hours later, and at the time of discharge if the post-infusion stay extends beyond 4 hours.

Determinants of the ICE score are:

- **Orientation:** Orientation to year, month, city, hospital: 1 point each for maximum of 4 points
- **Naming:** Name 3 objects (e.g., point to clock, pen, button): 1 point each for maximum of 3 points
- **Following 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:** Count backwards from 100 by ten: 1 point

Table 3: ASTCT Immune Effector Cell-Associated Neurotoxicity Syndrome Grading [a]				
Neurotoxicity Domain	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
ICE Score [b]	7–9	3–6	0–2	0 (subject is unarousable and unable to perform ICE.)
Depressed level of consciousness [c]	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Subjects is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma.
Seizure	N/A	N/A	Any clinical seizure Focal/generalized that resolves rapidly; or Non-convulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 minutes); or Repetitive clinical or electrical seizures without return to baseline in between.
Motor Findings [d]	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Raised ICP/ Cerebral Edema	N/A	N/A	Focal/local edema on neuroimaging [e]	Diffuse cerebral edema on neuroimaging; Decerebrate or Decorticate posturing; or Cranial nerve VI palsy; or Papilledema; or Cushing's triad

ASTCT, American Society for Transplantation and Cellular Therapy; CTCAE, Common Terminology Criteria for Adverse Events; ICANS, immune effector cell-associated neurotoxicity syndrome; ICE, immune effector cell-associated encephalopathy; ICP, intracranial pressure; EEG, electroencephalogram; N/A, not applicable.

[a] 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 subject with an ICE score of 3 who has a generalized seizure is classified as having Grade 3 ICANS.

[b] A subject with an ICE score of 0 may be classified as having Grade 3 ICANS if the subject is awake with global aphasia. But a subject with an ICE score of 0 may be classified as having Grade 4 ICANS if the subject is unarousable.

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

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

[e] 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.

Reference: Lee 2019.

Refer to Appendix IV for Management Guidelines.

8.9 Vascular Leak Syndrome

Neither administration of allogeneic NK cells nor autologous IL-2 activated NK infusions have been associated with vascular leak syndrome in our previous experience. Nevertheless, patients are monitored for weight gain and pulmonary edema during the dose limiting toxicity assessment period (through Day 28) and the retreatment period if applicable.

8.10 Enoblituzumab Adverse Events

The following were the most common side effects that were considered related to single-agent enoblituzumab administration and were seen in at least 1 of 10 adult participants. As of 13 April 2019, these side effects have been generally mild or moderate.

Very Common (≥10%)

- Infusion related reactions (IRRs) including CRS – Refer to Section 8.1
- Tiredness
- Nausea
- Chills
- Vomiting

A total of 11 patients have experienced serious side effects that were considered related to enoblituzumab. A serious side effect that occurred in 3 or more patients was infusion related reactions.

9 Clinical Evaluations and Procedures

Day 1 is equal to the day the 1st dose of FT516 is given. Day 1 is the anchor day for the study calendar. Although a cell infusion with lymphodepleting chemotherapy, there is no Day 0 in this calendar.

For Patient Cohorts 4 and 5 only: The 1st dose of enoblituzumab is given 1 to 2 days before the start of the cyclophosphamide/fludarabine. Subsequent doses are given every 3 weeks (±2 days) from the targeted dosing day.

A minimum of 48 hours must separate the last dose of fludarabine and the 1st dose of FT516. The 1st dose of FT516 may be delayed for up to 7 days after the last dose of fludarabine in the event FT516 cannot be given as planned. The 1st dose of FT516 equals Day 1.

A window of ±1 day for scheduling issues is permitted for Day 1, Day 8, and Day 15.

As detailed in Section 7.4, if on the day of the planned treatment, the study investigator feels it is not in the best interest to treat the patient, the FT516/IL-2 may be:

- Delayed for up to 48 hours adjusting future time points to maintain timing OR
- Skipped and the patient scheduled for the next planned visit. Skipped dose(s) are not made up.

Scheduled evaluations up to Day 29 (end of DLT period) may be performed ±1 days from the targeted date; assessments to be performed after Day 29 may be done ±3 days of the targeted date. In addition, targeted days may be altered as clinically appropriate.

Disease assessments are done per standard of care and for the purposes of follow-up disease and survival status the disease assessment closest to the targeted follow-up date should be used for month 3, 6, 9, and 12 follow-up time point. Note: these same time points apply to patients who are continuing enoblituzumab as disease status and survival are secondary endpoints.

9.1 Required Clinical Care Evaluations

	Screening[1] Within 28 days of registration	Prior to 1st dose of study drug	Enoblituzumab (Day -6) Level 4 and Level 5 only	CY/FLU		Day 1	Day 3	Day 8	Day 15	Day 22	Day 29 (end of DLT period)	Day 36	Day 43 and every 3 weeks for patients continuing on enoblituzumab	Dz assess every 3 months from Day 36 [5]	3, 6, 9 and 12 months from Day 1	End of Treat-ment Visit[10]
				#1 (Day -5)	#2 (Day -4)											
Consent	X[1]															
Screening Assessment	X															
Medical History	X											X				
Physical Exam	X										X		X			X
Provider Assessment			X	X	X	X	X	X	X	X		X				
Weight			X	X		X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X			X
Height	X															
Vitals and Pulse Oximetry	X		X			X[6]		X[6]	X[6]	X	X		X			
GOG Performance Status	X										X					
ICANS (neurotoxicity) monitoring)[7]	X		X			X[7]	X	X[7]	X[7]	X	X	X				
Toxicity Assessment (refer to Section 10.2 for AE documentation requirements)	X		X			X	X	X	X	X	X					
Survival Status															X	
Disease Response Status															X[5]	
CBC, diff, plt	X		X			X	X	X	X	X	X	X	X			X
Basic metabolic panel (BMP)[3]						X	X									
Comprehensive metabolic panel (CMP) or equivalent[4]	X		X	X				X	X	X	X	X	X			X
CRP. Ferritin, IL-6						Only if symptoms or diagnosis of CRS (refer to Section 8.6)											
CA-125	X											X		X		
Urine or serum pregnancy test [11]	X															
eGFR	X															
Disease staging by iRECIST	X											X		X		
CT or PET-CT of chest, abdomen and pelvis	X[1]											X		X		
Brain CT or MRI	X[2]															
PFTs	X[2]															
EKG	X															
Echocardiogram or MUGA or cardiac MRI	X															
IP catheter placement		X														
Enoblituzumab IV (COHORT 4 and LEVEL 5 Only)			R							R			R (continue every 3 weeks until PD)			
FT516/IL-2 IP						R		R	R				Re-treat[9]			

R= Research – do not charge to insurance

1 within 14 days for labs required for eligibility - For screening, prior disease assessments may be used if they were performed within 42 days of the 1st FT516 infusion in the absence of intervening anti-cancer therapy. Consent is exempt from 28 day limit - may be performed at any time prior to starting study related activity

2 perform only if known history or as medically indicated

3 basic metabolic panel consists of BUN, creatinine, calcium, glucose, lytes (CO2, Cl, Na, K)

4 comprehensive metabolic panel consists of albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, creatinine, glucose, electrolytes (CO2, Cl, Na,, K), total bilirubin, and total protein

5 Disease restaging every 3 months until disease progression or start of a new treatment then follow for survival only for 12 months from the 1st FT516. For any patient receiving FT516 Follow-up transferred to the LTFU protocol CPRC #2020LS072

6 Vital signs include oral temperature, systolic and diastolic blood pressure, heart rate, respiration rate, and pulse oximetry. On days of FT516 administration, collect as follows: within 15 minutes prior to infusion of FT516, at 10 (±5) minutes during infusion, and every 15 (±5) minutes for 1 hour following the end of infusion of the last administered bag of FT516. On days of enoblituzumab collect as follows: within 15 minutes of starting the enoblituzumab, every 15 minutes (± 5 minutes for the first hour of the infusion, every 30 minutes (±5 minutes) thereafter until infusion end, upon discontinuing infusion, and before the patient is discharged from the clinic

7 Neurotoxicity will be monitored using the ASTCT guidelines for grading ICANS based on the criteria in Section 8.8. ICANS monitoring will occur just prior to start of lympho-conditioning, at specified time points, and as needed in to document complete resolution to baseline status. **On FT516 dosing days, assessment for ICANS should be done prior to infusion, 2 to 4 hours after FT516 administration and at time of discharge the post-infusion extends beyond 4 hours.** Unscheduled assessments should be performed if new or worsening ICANS is suspected. In cases of documented changes in the ICANS grading, follow-up assessments should be performed until resolution to baseline or patient discontinuation from the study, whichever occurs earlier. Refer to Section 8.8 for details.

8 To monitor for vascular leak syndrome per Section 8.9

9 Re-treatment may be an option for patients with stable disease or better based on the Day 36 reassessment. Refer to Section 6.3 and Section 7.8 for additional information.

10 End of Treatment visit approximately 30 days (± 8 days) after the last dose of enoblituzumab (or FT516) whichever is later

11 Woman of childbearing potential and still have the anatomy (uterus and ovaries) to get pregnant must have a negative pregnancy test (urine or serum) within 14 days of study enrollment

9.2 Patient Research Related Evaluations

	Baseline (Prior to 1st dose of study drug)	Prior to enoblituzumab (Day -6) COHORT 4 and 5 ONLY	Prior to LD chemo	Day 1	Day 3	Day 8	Day 15	Day 22	Day 29	Day 36	Day 43	Research Sample Drawing Coincides with a SOC visit closest to the targeted time point			
												3 months from Day 1	6 months from Day 1	9 months from Day 1	12 months from Day 1
Enoblituzumab		X						X			X				
FT516 treatment				X		X	X								
ferritin, CRP (to FV lab per SOC) [1]	X			X					X						
Six 10 ml green top tubes – collect prior to the day's treatment (if applicable) [5]	X[5]	X	X	X	X	X	X	X	X	X	X	X (30 ml)	X (30 ml)	X (30 ml)	X (30 ml)
One 10 ml of red top tube – collect prior to the day's treatment (if applicable)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
PRA anti-HLA antibodies	X									X					
IP fluid (ascites preferred over washings) – collect prior to the day's treatment (if applicable) Refer to Section 9.2.1	At time of IP catheter placement			X		X	X	X	X[2]	X[3]	If retreat				
Tumor biopsy by IR (if feasible) 6 cores (5-10 mm) preferred but less is acceptable – to BioNet	At time of IP catheter placement									X[3]					
Safety baseline: 1 x 3 ml red top serum tube 1 x 10 ml green top tube – store frozen and batch ship to Fate	X														
Safety follow-up: 1 x 3 ml red top serum tube 1 x 10 ml green top tube 1 x 6 ml yellow top tube – store frozen and batch ship to Fate												X	X	(not collected at 9 months)	X

1 – Baseline ferritin and CRP are paid for by the study – if CRS occurs, additional ferritin and CRP levels are done as part of clinical care per Section 9.1 as detailed in 1st paragraph below this table

2- if NK cells are not present at Day 22, do not collect ascites or IP washing at Day 29

3 - at time of IP catheter removal (Day 36 if no re-treatment or at time of IP catheter removal if re-treatment)

4- Collect 5ml ascites-aliquot in 5 tubes minimum-200ul/vial, rest as 1ml/vial. Stored frozen for batching shipping to FATE for NK cell PKs

5 - At TTL: PBMCs are isolated from the heparin/green tube at pre-designated time points for PCR testing by Fate

Note: if a patient is not abiding by the required clinical care calendar (Section 9.1), the collection schedule of research related samples may be altered or discontinued on an individual patient basis, as appropriate. During follow-up no visit will be solely for research and instead be linked with a standard of care visit closest to the targeted research related timepoint.

All research samples go to the Masonic Cancer Center's Translational Therapy Lab (TTL) unless otherwise indicated. Baseline and Day 29 ferritin and CRP and PRA anti-HLA antibodies testing are charged to research but run in the treatment center's clinical lab. If additional ferritin and/or CRP levels are collected as part of good medical care (i.e. development of signs of CRS) they are to be charged as standard of care.

For all patients receiving FT516, samples as designated in the above table are shipped from TTL to Fate. For Cohorts 4 and 5 only, serum and ascites/IP wash samples are provided to MacroGenics by TTL to determine enoblituzumab levels.

It is recognized that with novel therapies as used in this study, the timing of protocol directed research samples may miss important patient specific events. For this reason, blood and/or IP fluid may be collected at up to 3 additional time points that are not specified above.

Samples to evaluate lymphocyte number and phenotype will be collected as detailed above for the Masonic Cancer Center Translational Therapy Lab (TTL) along with serum (red top tubes) for measure of cytokines that can reflect immune activation.

Flow cytometry analysis of a fraction of the PBMC will detect surface markers that define lymphocyte subsets (NK, NKT, B, and T cells, both CD4 and CD8), as well as intracellular markers that define regulatory T cells (Foxp3) and proliferating cells (Ki67). All remaining PBMC will be cryopreserved in 10% DMSO and stored in liquid nitrogen for future testing, if subject agreed to future storage at the time of initial consent.

Samples may be sent to laboratories outside of the University of Minnesota in cases where testing is not available internally as embedded in the patient consent form.

9.2.1 Intraperitoneal (IP) Fluid Collection

Ascites is preferred and a collection of such should be attempted first. It is recommended to obtain a minimum of 200cc and up to 500cc if ascites is present. Lower or higher volumes are acceptable. Any ascites collected, regardless of the volume, will be submitted to TTL as a separate sample from peritoneal washings if

both obtained. The higher the volume of ascites submitted, the better the yield of Tumor Ascites Lymphocytes (TALs).

If ascites is drained for clinical care at other times during this timeframe, a sample (up to 500 cc) may be submitted to TTL as an additional time point.

If no ascites is present or less than 50cc is collected, peritoneal washings will be performed. A volume of 250cc of room temperature NS will be infused into the abdomen. After infusion the patient will be asked to change position (right lateral, left lateral, Trendelenburg (feet higher than head by 30 degrees), reverse Trendelenburg) at 5-minute intervals to ensure adequate intra-abdominal distribution. Fifty (50) cc of infusate will then be retrieved through the indwelling catheter.

If a minimum of 50cc of infusate is not retrieved, another 250 cc of NS will be infused and the above process repeated. If after this 50cc of infusate have not been retrieved, the process will be terminated, however, any amount of infusate that is retrieved should be sent to TTL for testing.

9.2.2 Tumor Biopsies

Tumor biopsies will be performed if feasible. The inability or failure to do a biopsy will not be considered a protocol deviation. It is preferred that 6 cores (5-10 mm) be collected. It is understandable that it may not always be possible to obtain 6 cores and in such cases testing will be prioritized based on the number of cores received. The inability to collect any tumor samples or an insufficient amount will not be considered a protocol deviation.

Samples go to BioNet.

10 Event Monitoring, Documentation, and Reporting

For the purpose of the study FT516 and IL-2 are considered the investigational products for all patients. In addition, enoblituzumab is an investigational agent for patients treated in Cohort 4 or Cohort 5.

Adverse Reactions will be reported to the FDA per 21 CFR 312.32.

Toxicity and adverse events will be classified and graded according to NCI's Common Terminology Criteria for Adverse Events V 5.0 (CTCAE) and reported on the schedule below. A copy of the CTCAE can be downloaded from the CTEP home page.

(https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf)

An exception to the use of CTCAE will be for the assessment of cytokine release syndrome (CRS). Individual adverse events which are associated with CRS will be graded per CTCAE; however the ultimate assessment will be made using a revised grading system for CRS as presented by Lee et al (2019).

Table 4: ASTCT Cytokine Release Syndrome Consensus Grading System [a]

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever [b]	Temperature ≥38˚C	Temperature ≥38˚C	Temperature ≥38˚C	Temperature ≥38˚C
With either:				
Hypotension	None	Not requiring vasopressors	Requiring vasopressors with/with-out vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or [c]				
Hypoxia	None	Requiring low-flow nasal cannula [d] or blow-by	Requiring high-flow nasal cannula, facemask, non-rebreather mask, or venturi mask	Requiring positive pressure (e.g., CPAP, BiPAP, intubation and mechanical ventilation)

ASTCT, American Society for Transplantation and Cellular Therapy; BiPAP, bilevel positive airway pressure; CPAP, continuous positive airway pressure; CRS, cytokine release syndrome; NCI CTCAE, National Cancer Institute Common Terminology Criteria for Adverse Events.

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

[b] Fever is defined as temperature ≥38°C not attributable to any other cause. In subjects who have CRS then receive antipyretics or anti-cytokine 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.

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

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

Source: Lee et al. 2019.

The following definitions of adverse events (AEs) and serious adverse events (SAEs) will determine whether the event requires expedited reporting via the SAE Report Form in addition to routine documentation in the OnCore AE case report form (CRF).

10.1 Event Terminology

Adverse Event: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Suspected Adverse Reaction: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety

reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Adverse Reaction: Any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

Serious Adverse Event: An adverse event is considered "serious" if, in the view of either the investigator or IND Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Unexpected Event: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the protocol-related documents (e.g. protocol, consent documents, investigator brochure) or is not listed at the specificity or severity that has been observed or given the characteristics of the subject population being studied.

The following definitions are from the Masonic Cancer Center's Standard Operating Procedure (SOP) Deviation Reporting:

Major Deviation: A deviation or violation that impacts the risks and benefits of the research; may impact subject safety, affect the integrity of research data and/or affect a subject's willingness to participate in the research. Deviations that place a subject at risk, but do not result in harm are considered to be major deviations.

Minor Deviation: A deviation or violation that does not impact subject safety, compromise the integrity of research data and/or affect a subject's willingness to participate in the research.

10.2 Event Monitoring and Documentation Requirements

Monitoring for adverse events will begin with the insertion of the IP catheter through the removal of the IP catheter through the end of the dose limiting toxicity period (28 days after the 1st dose of FT516). Note: if a patient is not abiding by the planned treatment schedule, the documentation of adverse events may be altered or discontinued on an individual patient basis, as appropriate. During follow-up no visit will be solely for research and instead be linked with a standard of care visit closest to the targeted research related timepoint.

For patients receiving enoblituzumab maintenance (Cohort 4 and 5 only), AE monitoring continues through 28 days after the last dose or enoblituzumab.

The toxicity associated with the cyclophosphamide and fludarabine (CY/FLU) preparative regimen is well known and will not be documented unless the event meets the definition of a serious adverse event. Potential risks of CY/FLU are detailed in Appendix III.

Therefore, adverse event documentation for the purposes of this study will focus on

- Adverse events of special interest requiring targeted monitoring including infusion related reactions (IRR), neurotoxicity, vascular leak syndrome, and cytokine release syndrome as summarized in Table 5. Refer to Section 9.1 for timing/details.
- Any event within 28 days of the 1st FT516 infusion meeting the definition of dose limiting toxicity or for Step 1, an unacceptable toxicity per Section 10.3.
- Any event meeting the definition of a serious adverse event, regardless of attribution
- All Grade 3 and 4 adverse events regardless of attribution
- Grade 2 adverse events felt possibly, probably or related to either enoblituzumab or FT516

Table 5: Adverse Events of Special Interest Enoblituzumab and FT516/IL-2	
AE of Special Interest	**Monitoring summary**
Infusion related reaction to enoblituzumab	Refer to Section 7.2 for management of IRR - On days of enoblituzumab collect as follows: within 15 minutes of starting the enoblituzumab, every 15 minutes (± 5 minutes for the first hour of the infusion, every 30 minutes (±5 minutes) thereafter until infusion end, upon discontinuing infusion, and before the patient is discharged from the clinic
Infusion related reaction to FT516	Refer to Section 8.2 - Vital signs include oral temperature, systolic and diastolic blood pressure, heart rate, respiratory, and pulse oximetry. On days of FT516 administration, collect as follows: within 15 minutes prior to the infusion of FT516, at 10 (±5) minutes during infusion, and every 15 (±5) minutes for 1 hour following the end of infusion of the last administered bag of FT516
Cytokine Release Syndrome or CRS-Like Symptoms	Grade per Lee 2019 in Section 10 In the event of CRS obtain CRP and ferritin levels per Section 9.1
Neurotoxicity	Neurotoxicity will be monitored using the ASTCT guidelines for grading ICANS based on the criteria in Section 8.8. ICANS monitoring will occur just prior to start of lympho-conditioning, at specified time points, and as needed in to document complete resolution to baseline status. **On FT516 dosing days, assessment for ICANS should be done prior to infusion, 2 to 4 hours after FT516 administration and at time of discharge the post-infusion extends beyond 4 hours.** Unscheduled assessments should be performed if new or worsening ICANS is suspected. In cases of documented changes in the ICANS grading, follow-up assessments should be performed until resolution to baseline or patient discontinuation from the study, whichever occurs earlier.
Vascular Leak Syndrome	Potential risk of IL-2 – assessed by weight measures and signs of pulmonary edema

10.3 Dose Limiting Toxicity Event/Stopping Rule Documentation and Reporting

The following events require special documentation and reporting in addition to recording as an adverse event and reporting as a serious adverse event if that criteria is met.

10.3.1 During Fast-Track Enrollment Only

During Stage 1, Step 1 of fast-track enrollment only (cohorts of 1 patient), the 1st patient experiencing any one of the following adverse events within 28 days of the 1st FT516 dose triggers a change to 3 patients per cohort (Stage 1, Step 2). These definitions of unacceptable adverse events only are used for assessment in single patient cohorts.

- Abdominal pain that is unresponsive to management lasting more than 48 hours not attributable to disease status
- Grade 3 infusion related reaction (per CTCAE V5)

If a patent experiences a dose limiting toxicity (defined in next section), enrollment moves directly to Stage 2 (CRM).

10.3.2 Dose Limiting Toxicity (All Patients)

All patients are monitored for dose limiting toxicity (DLT). DLT is defined as any treatment emergent toxicity at least possibly related to the study treatment meeting one of the following criteria based on CTCAE v5 within 28 days (14 days for ascites) of the 1st FT516 infusion (for Cohort 4 and 5, DLT assessment starts with enoblituzumab and continues for 28 days after 1st FT516):

- Grade 3 organ toxicity (pulmonary, hepatic, renal, or neurologic) not pre-existing and lasting more than 72 hours
- Any non-hematologic Grade 4 or 5 toxicity
- Neutrophil count decreased ≥ Grade 4 that persists at Day 28 despite use of growth factor support
- Grade 3 or greater abdominal pain lasting more than 4 consecutive days and not controlled by standard analgesics
- Grade 3 or greater ascites within 14 days after FT516 administration in patients who had no ascites or Grade 1 ascites at enrollment and is not attributable to disease progression

In addition to documenting the event in the study's CRF's, all DLT are to be documented on the Event Form found in OnCore per Masonic Cancer Center procedures.

10.3.3 Early Stopping Rule Events (All Patients)

Stopping rules are in place during this study to stop the trial in case there is excessive toxicity. The following events must be reported within 24 hours of knowledge using the Stopping Rule form.

- Stopping Rule for Excessive DLT
- Infusion Related Reaction within 28 days of the 1st dose of FT516 (34 days after the 1st dose of enoblituzumab in Cohorts 4 and 5)
- Early Death (Grade 5 Event) within 28 days after the last dose of FT516 (21 days after Dose 2 of enoblituzumab in Cohorts 4 and 5) and not attributable to disease progression.

Any one death, suspends study enrollment per Section 13.4.

10.4 SAE and Death Documentation

Any event meeting the definition of a serious adverse event (SAE) requires documentation using the MCC SAE Report Form in OnCore.

Deaths, including due to disease within 1 year after the FT516 cell infusion will be recorded as an SAE. Deaths due to disease should be recorded as a Grade 5 "Neoplasms benign, malignant and unspecified (including cysts and polyps) – Other (Progressive Disease).

In addition, the death date and cause must be reported in the patient follow-up tab in OnCore using the comment field in the survival status section to record the cause.

10.5 Expedited Reporting Requirements

The following events require expedited reported:

Report to:	Criteria for reporting:	Timeframe:	Form to Use:	Submission email address
Advarra	unanticipated problems involving risks to subjects or others; unanticipated adverse device effects; protocol violations that may affect the subjects' rights, safety, or well-being and/or the completeness, accuracy and reliability of the study data; subject death; suspension of enrollment; or termination of the study	promptly and no later than 2 weeks (10 business days) from the time the investigator learns of the event	Refer to the Advarra IRB Handbook	Advarra via study specific CIRBI Link
UMN IRB	Refer to Submitting Updates in ETHOS – External IRB Study/Site			
FDA	Unexpected and fatal or unexpected and life threatening suspected adverse reaction	no later than 7 Calendar Days	MCC SAE Report Form	Submit to FDA as an amendment to IND with a copy to Fate Therapeutics at safety@fatetherapeutics.com, if Cohort 4 or 5 to MacroGenics saereports@MacroGenics.com
	1) Serious and unexpected suspected adverse reaction or 2) increased occurrence of serious suspected adverse reactions over that listed in the protocol or investigator brochure or 3) findings from other sources (other studies, animal or in vitro testing)	no later than 15 Calendar-Days		
	All other events per CFR 312.33	at time of annual report	IND annual report	
MacroGenics, Inc	For patients assigned to enoblituzumab: all SAEs, regardless of causality or expectedness (pregnancy N/A in this study)	within 7 calendar days of awareness	MCC SAE Report Form	saereports@MacroGenics.com
Fate Therapeutics	All SAEs	no later than 5 Calendar Days	MCC SAE Report Form	Fate Therapeutics at safety@fatetherapeutics.com
	Suspected Unexpected Serious Adverse Reaction (SUSAR)	No later than 3 Calendar Days		

The Masonic Cancer Center (MCC) SAE Coordinator receives an OnCore generated notification for any SAE, DLT, or stopping rule event. The MCC SAE Coordinator or designee is responsible for reviewing each report and determines if the event is reported in real time to the MCC Data and Safety Monitoring Council in real time or as part of quarterly summary report required for any high risk trial.

11 Investigational Products Information

11.1 Enoblituzumab

Enoblituzumab will be administered at a dose of 15 mg/kg, initially calculated based on the patient's actual weight prior to the 1st dose (baseline) measurement. Significant (≥ 10%) change in body weight from baseline should prompt recalculation of the dose. Refer to the Pharmacy Manual for further instructions on allowable parameters for dose rounding of enoblituzumab.

11.1.1 Availability

For the purposes of this study, enoblituzumab will be provided by MacroGenics.

11.1.2 Storage

Enoblituzumab will be stored in the University of Minnesota Investigational Drug Services (IDS) Pharmacy.

11.1.3 Preparation

The desired amount of enoblituzumab should be withdrawn from the vial(s) and diluted to the appropriate final concentration with 0.9% Sodium Chloride Injection USP (normal saline), according to the instructions provided in the Pharmacy Manual. A sterile, non-pyrogenic, low-protein binding polyethersulfone (PES) 0.2 micron in-line filter administration set must be used for IV administration of enoblituzumab. The infusion bag containing enoblituzumab should be gently inverted to mix the solution. The dose solution should be administered via IV infusion over 120 (± 15) minutes with a commercially available infusion pump."

11.1.4 Potential Toxicities

Refer to Section 8 for potential toxicities.

11.2 FT516

FT516 is an investigational product and can only be used and administered under an FDA approved protocol. For the purposes of this study FT516 is provided by Fate Therapeutics.

FT516 will be provided as a cryopreserved bag, thawed at the site of administration and administered by gravity without an in-line filter intraperitoneally via intraperitoneal catheter.

Refer to Section 8 for potential toxicities.

11.3 Interleukin-2

IL-2 is not approved for the indication and method of administration used in this study, although it has previously been given at the same dose intraperitoneally in previous studies performed at the University of Minnesota under IND 17568 (S/I Melissa A. Geller, MD). Dr. Geller provided a letter of authorization to in its review as part of the initial IND application for this study.

For the purposes of this study, IL-2 will be purchased by the University of Minnesota Fairview Investigational Drug Services (IDS) pharmacy. The IL-2 infusion will be prepared in IDS.

12 Study Data Collection and Monitoring

12.1 Data Management

This study will collect regulatory and clinical data using University of Minnesota CTSI's instance of OnCore® (Online Enterprise Research Management Environment). The Oncore database resides on dedicated secure and PHI compliant servers. The production server is located in the UMN datacenter (WBOB).

Additional immune monitoring data about correlative laboratory samples generated by the Masonic Cancer Center Translational Therapy Laboratory (TTL) from the protocol-directed correlative research samples is stored in their Laboratory Information Management System (LIMS).The LIMS database application is also stored on a production server located in the UMN datacenter (WBOB) and is managed by the Academic Health Center

Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore.

12.2 Case Report Forms

Participant data will be collected using protocol specific electronic case report forms (e-CRF) developed within OnCore based on its library of standardized forms. The e-CRF will be approved by the study's Principal Investigator and the Biostatistician prior to release for use. The Primary Clinical Research Coordinator or designee will be responsible for registering the patient into OnCore at time of study entry, completing e-CRF based on the patient specific calendar, and updating the patient record until patient death or end of required study participation.

12.3 Data and Safety Monitoring Plan (DSMP)

The Data and Safety Monitoring Plan of this study will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dsmp.

For the purposes of data and safety monitoring, this study is classified as high risk (under a locally held IND). Therefore the following requirements will be fulfilled:

- At least quarterly review of the study's progress by the Masonic Cancer Center Data and Safety Monitoring Council (DSMC).
- At least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per Section 10.5 to the Advarra, the FDA, Fate Therapeutics, and MacroGenics.

IND Annual Reports

In accordance with regulation 21 CFR § 312.33, the IND Sponsor (Dr. Geller) will submit a progress report annually. The report will be submitted within 60 days of the anniversary date that the IND went into effect.

12.4 Study Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the study's Principal Investigator and/or any designees, the IRB of record, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

12.5 Record Retention

Study records including source data, copies of case report forms, consent forms, HIPAA authorizations, and all study correspondence will be retained in a secured facility until permission is received that the documents are no longer needed.

In addition, the Clinical Trials Office (CTO) will keep a master log in OnCore of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO before destroying any study related records.

13 Statistical Considerations

13.1 Study Design, Objectives and Endpoints

This Phase I study will test up to 5 dose strategies of FT516 to determine the maximum tolerated dose (MTD) of FT516 with a maximum enrollment of 31 patients.

There are 5 potential levels defined for this study. The trial will be conducted with no intra-patient escalation. The starting dose will be dose $9x10^7$ FT516 cells/dose with no enoblituzumab. The subsequent planned cohorts will be $3x10^8$ FT516 cells/dose with no enoblituzumab, and then $9x10^8$ FT516 cells/dose with no enoblituzumab. Dose escalation will continue in combination with enoblituzumab starting at 1 dose below the highest dose reached from the 1st 3 cohorts given that we reach at least Cohort 2 without enoblituzumab. This will potentially continue up through $9x10^8$ FT516 cells/dose + enoblituzumab. Given that little to no toxicity is expected, the MTD will be determined using an adaptation of the continual reassessment method (CRM) (O'Quigley, 1996). A cohort of 1 patient will start at Cohort 1 with subsequent planned cohorts as described in Table 6:

Table 6. Infuse FT516 cell product at the assigned dose on Day 1, Day 8 and Day 15

Cohort	FT516 Dose IP (cells per dose)
1	Monotherapy: IP FT516 at 9 x 10^7 cells/dose on Day 1, 8, and 15
2	Monotherapy: IP FT516 at 3 x 10^8 cells/dose on Day 1, 8, and 15
3	Monotherapy: IP FT516 at 9 x 10^8 cells/dose on Day 1, 8, and 15
4	Safe FT516 dose (MTD-1) from 1st 3 levels + IV enoblituzumab on Day -6
5	Highest FT516 dose (MTD) from 1st 3 levels + IV enoblituzumab on Day -6

The Phase I trial will be conducted in two consecutive stages: Stage 1 Step 1 will enroll one patient at consecutively increasing doses until any of the following adverse events occurs within 28 days of the 1st dose of FT516:

- Abdominal pain that is unresponsive to management lasting more than 48 hours not attributable to disease status
- Grade 3 infusion reaction (per CTCAE V5)

At this point Stage 1, Step 2 is initiated and the cohort size will increase from 1 to 3 patients. Note: if a DLT occurs during Fast-track (Step 1), enrollment moves directly to Stage 2 and Step 2 is not used.

At the 1st dose-limiting toxicity (DLT, defined in Section 7.5) occurs, Stage 2 of the Phase I trial will be initiated. In this stage, the dose escalation will be based on a simple application of the likelihood version of the CRM. A one-parameter model has been chosen to link the risk of a DLT to the dose, where the probability of toxicity at dose i is modeled as $p_i^{\exp(\alpha)}$ where pi is a constant and α is the parameter to be estimated. The goal will be to identify one of the 5 dose level strategies corresponding to the desired maximum toxicity rate of ≤25%.

Given that we will have prior data once the CRM is initiated, no “skeleton” estimates are needed. If the CRM is initiated, the CRM will continually update the toxicity at the end of 28 days after the 3rd patient in each cohort. Each new cohort of three patients will be sequentially assigned to the most appropriate dose based on the updated toxicity probabilities. The MTD will be identified by the minimum of the following criteria: (1) the total sample size of 25 is exhausted, (2) 10 consecutive patients are enrolled at the same dose or (3) the probability that the next 5 patients will be allocated to the same dose-level, based on the current estimate of the probability of toxicity, exceeds 90%. (Zohar 2001) The function ‘crm’ from the R package ‘dfcrm’ will calculate posterior means of toxicity probabilities. Dose escalation of more than one level is not permitted with this design.

13.2 Sample Size

A maximum of 31 patients will be enrolled. Based on the simulations from Table 6 and Table 7, this should be sufficient and safe to define the MTD.

Table 7. Operating characteristics for Adaptive-CRM

Cells/ dose	Expected DLT			Excessive DLT		
	True Probability	Prob. of dose	N	True Probability	Probability of dose	N
9 x 10^7 alone	1%	0%	1	15%	32%	6
3 x 10^8 alone	3%	0%	1	30%	52%	12
9 x 10^8 alone	6%	0%	1	45%	16%	6
Safe dose from 1st 3 levels(MTD-1) +enoblituzumab	8%	0%	1	60%	0%	1
Highest dose from 1st 3 levels (MTD) + enoblituzumab	10%	100%	10	70%	0%	0

Enrollment will most likely include 14 patients, 22 patients if adverse events are encountered initially, or it could be as high as 31 patients if DLTs are encountered early. Accrual should range from 10-12 patients per year so study accrual is expected to be complete within 24-36 months.

Any patient who does not receive at least 1 dose of FT516 will be replaced to complete enrollment at a specified dose (e.g. 10 evaluable patients at the MTD).

13.3 Statistical Analysis

The primary objective of this trial is to identify the maximum tolerated dose (MTD) which will be determined per the study design and consultation with the study statistician using R prior to each cohort enrollment. Due to small patient numbers at the MTD, estimation of toxicity rates, clinical activity of progression free survival (PFS) at 12 months will be estimated in a descriptive format using simple frequencies, proportions, means, standard deviations/standard errors, medians and ranges and respective plots. Potential censored data such as PFS and relapse/progression and NRM may be estimated by Kaplan-Meier curves at 12 months. Relapse/progression and NRM may be estimated by cumulative incidence, treating NRM and relapse as competing risks, respectively.

13.4 Monitoring Guidelines (Early Study Stopping Rules)

Stopping rules also are in place independent of dose escalation. (Ivanova 2005).

Stopping Rule for Excessive DLT
A stopping rule is in place during the Phase I study to stop the trial in case there are excessive DLTs as defined by updated posterior probabilities throughout the trial. At the end of the 28 day evaluation period after each cohort of patients is enrolled, new posterior probabilities will be calculated for each dose. The trial will be stopped if the posterior probability that the lowest dose is unacceptably toxic (> 25% of patients) is greater than 80%.

Infusion Related Reaction within 28 Days of the 1st Dose of FT516 (34 days after the 1st dose of enoblituzumab in Cohorts 4 and 5)
The goal is to construct a boundary based on Grade 3 or greater infusion related reaction such that the probability of early stopping is at most 10% if the rate is equal to 5% and our sample size is at most 31. With these stipulations, the trial will be stopped and reviewed if 2/7, 3/16, 4/28 or 5 patients have events by Day 28. If the true probability of infusion related toxicity is 20%, there is an 88% chance of triggering the monitoring boundary.

Early Death (Grade 5 Event) within 28 days after the last dose of FT516 (21 days after Dose 2 of enoblituzumab in Cohorts 4 and 5)
Enrollment will be suspended and reviewed by the study team with follow-up notification to the FDA and IRB of the findings before enrollment is restarted for any death within 28 days after the last dose of FT516 (21 days after Dose 2 of enoblituzumab in Cohorts 4 and 5) and not attributable to disease progression.

14 Ethical and Regulatory Considerations

14.1 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

14.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in

order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

14.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved Consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, she will be asked to sign and date the Consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

15 References

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Appendix I – GOG PS Scale and NYHA Classification

GOG Score	Activity Level
0	Fully active, unrestricted activities of daily living
1	Ambulatory, but restricted in strenuous activity
2	Ambulatory, and capable of self care. Unable to work. Out of bed for greater than 50% of waking hours
3	Limited self care, or confined to bed or chair 50% of waking hours. Needs special assistance
4	Completely disabled, and no self care
5	Dead

NYHA Class	Patients with Cardiac Disease (Description of HF Related Symptoms)
Class I (Mild)	Patients with cardiac disease but without resulting in limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation (rapid or pounding heart beat), dyspnea (shortness of breath), or anginal pain (chest pain).
Class II (Mild)	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain
Class III (Moderate)	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.
Class IV (Severe)	Patients with cardiac disease resulting in the inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.

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Appendix II - iRECIST version 1.1

Modified Response Evaluation Criteria in Solid Tumors (RECIST), Version 1.1 for Immune-Based Therapeutics (iRECIST)

1 DEFINITIONS OF DISEASE

1.1 Measurable Disease

Measurable lesions are defined as those that can be accurately measured in ≥1 dimension (longest diameter to be recorded) as >10 mm with computed tomography (CT) scan (with minimum slice thickness of 5 mm), or >10 mm caliper measurement by clinical exam, or >20 mm by chest X-ray.

Pathological lymph nodes may also be considered as target on-target lesions. To be considered pathologically enlarged and measurable (target lesion), a lymph node must be ≥15 mm in short axis when assessed by CT scan (minimum slice thickness of 5 mm). Lymph nodes with a short axis ≥10 mm but <15 mm should be considered non-target lesions. Lymph nodes that have a short axis <10 mm are considered nonpathologic and should not be recorded as target lesions at baseline.

1.2 Nonmeasurable Disease

Nonmeasurable disease comprises all other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph notes with ≥10 mm but <15 mm short axis) as well as leptomeningeal disease, ascites, pleural or pericardial effusions, inflammatory breast disease, lymphangitis cutis or pulmonis, abdominal masses, or organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.3 Other Disease

Bone lesions: Bone scan, positron emission tomography (PET) scan or plain films are not considered adequate imaging techniques to measure bone lesions. However, these techniques can be used to confirm the presence or disappearance of bone lesions. Lytic bone lesions or mixed lytic-blastic lesions, with identifiable soft tissue components, that can be evaluated by cross-sectional imaging techniques such as CT or MRI can be considered as measurable lesions if the soft-tissue component meets the definition of measurability described in Section 1.1 above. Blastic bone lesions are considered nonmeasurable.

Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable or nonmeasurable) since they are, by definition, simple cysts. Cystic lesions thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described in Section 1.1 above. However, if non-cystic lesions are present in the same subject, these are preferred for selection as target lesions.

Tumor lesions situated in a previously irradiated area, or in an area subjected to other locoregional therapy, are not considered measurable unless there has been demonstrated progression in the lesion. Such lesions should not be selected as target lesions when other measurable lesions are available.

2 DEFINITIONS OF TARGET AND NON-TARGET LESIONS

2.1 Target Lesions

Up to a maximum of 5 measurable lesions total (and a maximum of 2 lesions per organ) representative of all involved organs should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate and reproducible repeated measurements (either by imaging techniques or clinically).

2.2 Non-target Lesions

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements are not required and these lesions should be followed as "present", "absent" or "unequivocal progression." Non-target lesions include measurable lesions that exceed the maximum number per organ or total of all involved organs as well as nonmeasurable lesions. It is possible to record multiple non-target lesions involving the same organ as a single item on the case report form (e.g., "multiple enlarged pelvic lymph nodes" or "multiple liver metastases"). Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

3 GUIDELINES FOR EVALUATION OF DISEASE

3.1 Methods of Assessment

CT is the best currently available and reproducible method to measure lesions selected for response assessment. MRI is also acceptable in certain situations (e.g., for body scans). The minimum slice thickness should be 5 mm. If slice thickness is <5 mm, the minimum size for a measurable lesion should be twice the slice thickness.

The utility of other methods other than CT or MRI to assess response are summarized as follows:

- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.
- Chest X-ray may be used to follow measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT of lesions of the chest is preferred.
- When available, functional fluorodeoxyglucose (FDG)-PET data can be used to complement CT data when assessing PD but is not a formal component of disease assessment for solid tumors.
- Ultrasound should not be used as a method of assessment.
- The use of endoscopy or laparoscopy for objective tumor evaluation is not advised. However, these techniques can be useful to confirm complete pathological response when biopsies are obtained or to determine disease relapse.
- Tumor markers can be followed but cannot be used alone to assess response or progression. If markers are initially above the upper normal limit, they must normalize for a subject to be considered in CR.
- Cytology or histology can be used to differentiate between PR and CR in rare cases (e.g., residual lesions in tumor types, such as germ-cell tumors, where known residual benign tumors can remain). Because an effusion may be a side effect of some treatments, the

cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment is mandatory to differentiate between CR, PR, SD, and PD.

3.2 Reproducibility of Assessment

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Ideally, the same individual should consistently perform assessments.

Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment. To ensure comparability, the baseline radiology/scans and subsequent radiology/scans to assess response should be performed using identical techniques whenever possible (e.g., scans performed immediately following bolus contrast administration should be made with a standard volume of contrast, the identical contrast agent, and the same scanner).

3.3 Determination of Tumor Response and Progression

Determination of tumor responses and disease progression should follow the following guidelines:

- All baseline evaluations should be performed as closely as possible to the beginning of treatment within the protocol-defined screening period.
- All sites of disease will be followed as either target or non-target lesions, as categorized at baseline. All measurable lesions up to a maximum of 2 lesions per organ or 5 lesions in total, representative of all involved organs, should be identified as target lesions, while all other lesions (either additional measurable lesions or nonmeasurable lesions) will be classified as non-target lesions.
- All measurements will be taken and recorded in metric notation using a ruler or calipers. A sum of the diameters for all target lesions will be calculated and reported as the baseline sum of the diameters. For solid tumor lesions, only the long axis is added to the sum and for lymph nodes, only the short axis is added to the sum.
- All lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g., 2 mm). If the lesion has likely disappeared, the measurement should be recorded as 0 mm. If a target lesion (nodal or non-nodal) becomes so faint on radiographic imaging that an exact measurement cannot be assigned, then a default value of 5 mm (minimum slice thickness) should be assigned.
- The short axis measurement of any lymph node that is considered a target lesion should continue to be recorded even if the node regresses to <10 mm. However, because this may prevent the sum of lesions from being zero even if CR criteria are met, target lymph nodes that regress to <10 mm can be considered to have become normal for purposes of CR calculation.
- At each post-baseline tumor assessment, the sum of the diameters of the index lesions will be added together to calculate the sum of target lesions. Comparison of subsequent assessments to the smallest sum of the diameters (nadir tumor burden), including the baseline sum if that is the smallest sum of the diameters during the study, will be used to characterize objective tumor progression in the measurable dimensions of the disease.
- New lesions should be assessed and categorized as measurable or nonmeasurable. Any new, measurable lesions (as defined in Section 1.1 above) (up to a maximum of 5 measurable new lesions total [2 new lesions per organ]) representative of all involved organs, will be measured

and recorded separately on the case report form but not included in the sum of lesions for target lesions identified at baseline. Other measurable and nonmeasurable lesions will be recorded as new lesions non-target. If a new lesion is identified (thus meeting the criteria for iUPD) and the subject is clinically stable, treatment should be continued. New lesions do not have to resolve for subsequent iSD or iPR providing that the next assessment did not confirm progression. New lesions do not need to meet the criteria for new target lesion to result in iUPD (or iCPD). New lesions that are either target or non-target can drive iUPD or iCPD. Progressive disease is confirmed (iCPD) in the new lesion category if the next imaging assessment confirms additional new lesions or a further increase in new lesion size from iUPD (sum of measures increase in new target lesion ≥5 mm, any increase for new non-target lesions).

4 TUMOR RESPONSE AND PROGRESSION CRITERIA

Responses will be categorized as iCR, iPR, iSD, iUPD, or iCPD. In addition, a response category of non-evaluable (NE) is provided for situations in which there is inadequate information to otherwise categorize response status.

4.1 Target Lesions

The definitions of response and progression for target lesions is:

- iCR: Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.
- iPR: At least a 30% decrease in the sum of diameters of target lesions, taking as reference the Screening (baseline) sum diameters.
- iUPD: At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the Screening (baseline) sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. iUPD can be assigned multiple times as long as iCPD is not confirmed at the next assessment.
- iCPD: if the target lesion response was iUPD at the last timepoint and shows a further increase in tumor burden as evidenced (as applicable) by a ≥5 mm increase in sum of measures of target lesions. However, the criteria for iCPD (after iUPD) are not considered to have been met if iCR, iPR, or iSD criteria (compared with baseline and as defined by RECIST 1.1) are met at the next assessment after iUPD. The status is reset and iCR, iPR, or iSD should then be assigned; and if no change is detected, then the timepoint response is iUPD.
- iSD: Neither sufficient shrinkage to qualify for iPR nor sufficient increase to qualify for iUPD.
- NE: In a subject who does not have iUPD or iCPD, the inability to perform a response assessment due to missing data regarding target lesions.

4.2 Non-target Lesions

While some non-target lesions may be measurable, they need not be measured and instead should be assessed only qualitatively at the timepoints specified in the protocol. The definitions of response and progression for non-target lesions are:

- iCR: Disappearance of all non-target lesions, normalization of an elevated tumor marker level, and all lymph nodes nonpathologic in size (<10 mm in the short axis).

- Non-iCPD/non-iUPD: Persistence of ≥1 non-target lesion and/or maintenance of tumor marker level above the ULN.
- iUPD: Unequivocal progression of existing non-target lesion representing substantial worsening in non-target disease such that, even in the presence of stable or decreasing target disease, the overall tumor burden appears to have increased. iUPD can be assigned several times as long as iCPD is not confirmed at the next assessment.
- iCPD: Progressive disease in the non-target lesion category is confirmed if subsequent imaging shows a further increase from iUPD. The criteria for iCPD are not judged to have been met if RECIST 1.1 criteria for complete response or non-iCR/non-iUPD are met after a previous iUPD. The status is reset and iCR or non-iCR/non-iUPD is assigned. If no change is detected, the timepoint response is iUPD.
- NE: In a subject who does not have iUPD or iCPD, the inability to perform a response assessment due to missing data regarding non-target lesions.

4.3 Determination of Response or Progression at Each Timepoint

The occurrence of tumor response or progression will be determined at each timepoint. The table below provides a summary of the overall response or progression status at each timepoint.

Assignment of Timepoint Response Using iRECIST				
Target Lesions [a]	**Non-target Lesions [a]**	**New Lesions [a]**	**Time Point Response**	
			No prior iUPD [b]	**Prior iUPD [b, c]**
iCR	iCR	No	iCR	iCR
iCR	Non-iCR/ Non-iUPD	No	iPR	iPR
iPR	Non-iCR/ Non-iUPD	No	iPR	iPR
iSD	Non-iCR/ Non-iUPD	No	iSD	iSD
iUPD with no change OR decrease from last timepoint	iUPD with no change OR decrease from last timepoint	Yes	N/A	New lesions confirm iCPD if new lesions were previously identified and increase in size (≥5 mm in SOM for new target lesions or any increase for new non-target lesions) or number. If no change in new lesions (size or number) from the last timepoint, remains iUPD.
iSD, iPR, iCR	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in size of non-target disease (need not meet RECIST 1.1 criteria for unequivocal PD).
iUPD	Non-iCR/ Non-iUPD, or iCR	No	iUPD	Remains iUPD unless iCPD confirmed based on a further increase in SOM of at least 5 mm, otherwise remains iUPD.
iUPD	iUPD	No	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: • Previously identified target lesion iUPD SOM ≥5 mm and/or • Non-target lesion iUPD (prior assessment – need not be unequivocal PD)
iUPD	iUPD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on further increase in: • Previously identified target lesion iUPD SOM ≥5 mm and/or • Previously identified non-target lesion iUPD (need not be unequivocal) and/or • Size or number of new lesions previously identified
Non-iUPD/PD	Non-iUPD/PD	Yes	iUPD	Remains iUPD unless iCPD confirmed based on increase in size or number of new lesions previously identified.

iCPD, confirmed progressive disease; iCR, complete response; iPR, partial response; iSD, stable disease; iUPD, unconfirmed progressive disease; N/A, not applicable; SOM, sum of measures.

[a] Using RECIST 1.1 principles. If no PD occurs, RECIST 1.1 and iRECIST categories for CR, PR, and SD would be the same.

[b] In any lesion category.

[c] Previously identified in assessment immediately prior to this timepoint.

Reference: Seymour et al. 2017.

4.4 Best Overall Response and Confirmation of Tumor Status

For iRECIST, the best overall response (iBOR) recorded from the start of treatment until the end of treatment will be determined. iUPD will not override a subsequent best overall response of iSD, iPR, or iCR, meaning that iPR or iSD can be assigned even if new lesions have not regressed, or if unequivocal progression (non-target lesions) remains unchanged, providing that the criteria for iCPD are not met. iCR or iPR must be confirmed at a second tumor assessment obtained at >4 weeks from the assessment at which iCR or iPR was first observed. Confirmation requirements for each successive set of 2 scans in determining iBOR are provided the table below.

Best Overall iRECIST Response Assessment Considering Requirement for Confirmation		
Response Category at First Timepoint	**Response Category at Subsequent Timepoint**	**Best Overall Response**
iCR	iCR	iCR
iCR	iPR	iUPD unless minimum criteria for iSD met, [a] in which case iSD (possible iPR [b])
iCR	iSD	iSD if minimum criteria for iSD met, [a] otherwise iUPD if no further assessment
iCR	iUPD	iSD if minimum criteria for iSD met, [a] otherwise iUPD if no further assessment
iCR	NE	iSD if minimum criteria for iSD met, [a] otherwise NE
iPR	iCR	iPR
iPR	iPR	iPR
iPR	iSD	iSD
iPR	iUPD	iSD if minimum criteria for iSD met, [a] otherwise iUPD if no further assessment
iPR	NE	iSD if minimum criteria for iSD met, [a] otherwise NE
NE	NE	NE

iBOR, best overall response; iCR, complete response; NE, not evaluable; iPR, partial response; iSD, stable disease; iUPD, unconfirmed progressive disease.

[a] Assignment of iSD requires that at least one post-baseline scan was obtained at least 4 weeks from start of study therapy and met criteria for iSD or better response.

[b] If a true iCR occurs at the first timepoint, then any disease seen at a subsequent timepoint (even disease meeting PR criteria relative to baseline) results in a iBOR of iUPD (because the disease must have reappeared after the iCR). The BOR assessment would depend on whether the minimum duration for iSD was met. For a subject who retrospectively had only an apparent iCR (with small lesions still present, ie the subject had iPR, not iCR) at the first timepoint, the original iCR should be converted to an iPR and the iBOR should be assessed as iPR.

Reference: Eisenhauer et al. 2009.

Appendix III – Expected Toxicities - Preparative Regimen and IL-2

Cyclophosphamide		
Common	**Less Common**	**Rare, but may be serious**
• low white blood cell count with increased risk of infection • hair loss or thinning, including face and body hair (usually grows back after treatment) • nausea • vomiting • loss of appetite • sores in mouth or on lips • bleeding from bladder, with blood in urine • diarrhea • long-term or short-term infertility (inability to have children) in women and men	• low platelet count (mild) with increased risk of bleeding • darkening of nail beds • acne • tiredness • infection • fetal changes if you become pregnant while taking cyclophosphamide	• heart problems with high doses, with chest pain, shortness of breath, or swollen feet • severe allergic reactions • skin rash • scarring of bladder • kidney damage (renal tubular necrosis) which can lead to kidney failure • heart damage, with trouble getting your breath, swelling of feet, rapid weight gain • scarring of lung tissue, with cough and shortness of breath • second cancer, which can happen years after taking this drug • death from infection, bleeding, heart failure, allergic reaction, or other causes

Fludarabine		
Common	**Less Common**	**Rare, but may be serious**
• low white blood cell count with increased risk of infection • low platelet count with increased risk of bleeding • low red blood cell count (anemia) with tiredness and weakness • tiredness (fatigue) • nausea • vomiting • fever and chills • infection	• pneumonia • diarrhea • loss of appetite • weakness • pain	• numbness and tingling in hands and/or feet related to irritation of nerves • changes in vision • agitation • confusion • clumsiness • seizures • coma • cough • trouble breathing • intestinal bleeding • weakness • death due to effects on the brain, infection, bleeding, severe anemia, skin blistering, or other causes

Interleukin-2 (IL-2)		
Common	**Less Common**	**Rare, but may be serious**
• fever and chills or flu-like symptoms • generalized flushing (redness) of the face and body, or skin rash. • nausea or vomiting • lowered blood pressure • diarrhea • low blood counts increasing the risk of infection, anemia and//or bleeding • changes in mental status, such as confusion, drowsiness or memory loss • fast heartbeats • lowered urine output • changes in liver function • generalized aches and pains • swelling of the face, ankles or legs	• respiratory congestion or breathing difficulty • itching • mouth sores • poor appetite • fatigue • weight gain or loss • Infection • irregular heartbeats • dizziness • dry or peeling skin • injection site reactions	• capillary leak syndrome

Appendix IV – Management of Clinical Neurotoxicity

Management of clinical neurotoxicity, i.e., encephalopathy syndrome, status epilepticus, and raised intracranial pressure, should follow current recommendations for CAR-T-cell therapies (Neelapu 2018; Table A-1, Table A-2, and Table A-3) and/or institutional practice.

Table A-1 Recommendations for the Management of Encephalopathy Syndrome

Grade	Management
Grade 1	• Vigilant supportive care; aspiration precautions; IV hydration • Withhold oral intake of food, medicines, and fluids, and assess swallowing • Convert all oral medications and/or nutrition to IV if swallowing is impaired • Avoid medications that cause central nervous system depression • Low doses of lorazepam (0.25–0.5 mg IV every 8 hours) or haloperidol (0.5 mg IV every 6 hours) can be used, with careful monitoring, for agitated subjects • Neurology consultation • Fundoscopic exam to assess for papilloedema • MRI of the brain with and without contrast; diagnostic lumbar puncture with measurement of opening pressure; MRI spine if the subject has focal peripheral neurological deficits; CT scan of the brain can be performed if MRI of the brain is not feasible • Daily 30-minute EEG until toxicity symptoms resolve; if no seizures are detected on EEG, continue levetiracetam 750 mg every 12 hours • If EEG shows non-convulsive status epilepticus, treat as per algorithm in Table A-2 • Consider anti-IL-6 therapy with tocilizumab 8 mg/kg [a] IV or siltuximab 11 mg/kg IV, if encephalopathy is associated with concurrent CRS
Grade 2	• Supportive care and neurological work-up as described for grade 1 encephalopathy • Tocilizumab 8 mg/kg [a] IV or siltuximab 11 mg/kg IV if associated with concurrent CRS • Dexamethasone 10 mg IV every 6 hours or methylprednisolone 1 mg/kg IV every 12 hours if refractory to anti-IL-6 therapy, or for encephalopathy without concurrent CRS • Consider transferring subject to ICU if encephalopathy associated with Grade ≥2 CRS
Grade 3	• Supportive care and neurological work-up as indicated for Grade 1 encephalopathy • ICU transfer is recommended • Anti-IL-6 therapy if associated with concurrent CRS, as described for Grade 2 encephalopathy and if not administered previously • Corticosteroids as outlined for Grade 2 encephalopathy if symptoms worsen despite anti-IL-6 therapy, or for encephalopathy without concurrent CRS; continue corticosteroids until improvement to Grade 1 encephalopathy and then taper • Stage 1 or 2 papilloedema with CSF opening pressure <20 mmHg should be treated as per algorithm presented in Table A-3 • Consider repeat neuroimaging (CT or MRI) every 2–3 days if subject has persistent grade ≥3 encephalopathy
Grade 4	• Supportive care and neurological work-up as outlined for Grade 1 encephalopathy • ICU monitoring; consider mechanical ventilation for airway protection • Anti-IL-6 therapy and repeat neuroimaging as described for Grade 3 encephalopathy • High-dose corticosteroids continued until improvement to Grade 1 encephalopathy and then taper; for example, methylprednisolone IV 1 g/day for 3 days, followed by rapid taper at 250 mg every 12 hours for 2 days, 125 mg every 12 hours for 2 days, and 60 mg every 12 hours for 2 days • For convulsive status epilepticus, treat as per algorithm in Table A-2 • Stage ≥3 papilloedema, with a CSF opening pressure ≥20 mmHg or cerebral oedema, should be treated as per algorithm in Table A-3

CAR, chimeric antigen receptor; CSF, cerebrospinal fluid; CRS, cytokine release syndrome; CT, computed tomography (scan); EEG, electroencephalogram; ICU, intensive care unit; IV, intravenous; MRI, magnetic resonance imaging.

[a] Maximum amount of tocilizumab per dose is 800 mg.

Table A-1 Recommendations for the Management of Encephalopathy Syndrome	
Grade	**Management**

Reference: Neelapu 2018.

Table A-2 Recommendations for the Management of Status Epilepticus	
Status Epilepticus Type	**Management**
Non-convulsive status epilepticus	• Assess airway, breathing, and circulation; check blood glucose • Lorazepam [a] 0.5 mg IV, with additional 0.5 mg IV every 5 minutes, as needed, up to a total of 2 mg to control electrographical seizures • Levetiracetam 500 mg IV bolus, as well as maintenance doses • If seizures persist, transfer to ICU and treat with phenobarbital loading dose of 60 mg IV • Maintenance doses after resolution of non-convulsive status epilepticus are as follows: lorazepam 0.5 mg IV every 8 hours for three doses; levetiracetam 1,000 mg IV every 12 hours; phenobarbital 30 mg IV every 12 hours
Convulsive status epilepticus	• Assess airway, breathing, and circulation; check blood glucose • Transfer to ICU • Lorazepam [a] 2 mg IV, with additional 2 mg IV to a total of 4 mg to control seizures • Levetiracetam 500 mg IV bolus, as well as maintenance doses • If seizures persist, add phenobarbital treatment at a loading dose of 15 mg/kg IV • Maintenance doses after resolution of convulsive status epilepticus are: lorazepam 0.5 mg IV every 8 hours for three doses; levetiracetam 1,000 mg IV every 12 hours; phenobarbital 1–3 mg/kg IV every 12 hours • Continuous electroencephalogram monitoring should be performed, if seizures are refractory to treatment

ICU, intensive care unit; IV, intravenous.

NOTE: All indicated doses of medication are for adult subjects.

[a] Lorazepam is the recommended benzodiazepine because it is short-acting, compared with diazepam, and has been widely used in the management of seizures.

Reference: Neelapu 2018.

Table A-3 Recommendation for the Management of Raised Intracranial Pressure (ICP)	
Stage	**Management**
Stage 1 or 2 papilledema [a] with CSF opening pressure of <20 mmHg without cerebral edema	• Acetazolamide 1,000 mg IV, followed by 250–1,000 mg IV every 12 hours (adjust dose based on renal function and acid-base balance, monitored 1–2 times daily)
Stage 3, 4, or 5 papilloedema, [a] with any sign of cerebral oedema on imaging studies, or a CSF opening pressure of ≥20 mmHg	• Use high-dose corticosteroids with methylprednisolone IV 1 g/day, as recommended for Grade 4 encephalopathy syndrome (Table A-1) • Elevate head end of the subject's bed to an angle of 30 degrees • Hyperventilation to achieve target partial pressure of arterial carbon dioxide ($PaCO_2$) of 28–30 mmHg, but maintained for no longer than 24 hours • Hyperosmolar therapy with either mannitol (20 g/dL solution) or hypertonic saline (3% or 23.4%, as detailed below) – Mannitol: initial dose 0.5–1 g/kg; maintenance at 0.25–1 g/kg every 6 hours while monitoring metabolic profile and serum osmolality every 6 hours, and withhold mannitol if serum osmolality is ≥320 mOsm/kg, or the osmolality gap is ≥40 – Hypertonic saline: initial 250 mL of 3% hypertonic saline; maintenance at 50–75 mL/h while monitoring electrolytes every 4 hours, and withhold infusion if serum Na levels reach ≥155 mEq/L – For subjects with imminent herniation: initial 30 mL of 23.4% hypertonic saline; repeat after 15 minutes, if needed • If subject has ommaya reservoir, drain CSF to target opening pressure of <20 mmHg • Consider neurosurgery consultation and IV anesthetics for burst-suppression pattern on electroencephalography • Metabolic profiling every 6 hours and daily CT scan of head, with adjustments in usage of the aforementioned medications to prevent rebound cerebral edema, renal failure, electrolyte abnormalities, hypovolemia, and hypotension

CSF, cerebrospinal fluid; CT, computed tomography (scan); IV, intravenous.
NOTE: All medication doses indicated are for adults.
[a] Papilledema grading should be performed according to the modified Frisén scale.

Reference: Neelapu 2018.