# PROTOCOL AMENDMENT #3

**LCCC 1541-ATL:** Administration of autologous CAR-T cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch in subjects with Acute Lymphoblastic Leukemia

**AMENDMENT INCORPORATES (check all that apply):**

X Editorial, administrative changes
X Scientific changes (IRB approval)
Therapy changes (IRB approval)
Eligibility Changes (IRB approval)

The protocol is amended to shorten the DLT/safety assessment period from 6 weeks to 4 weeks. The protocol is also revised to include updated information on AP1903 and to provide other administrative/editorial revisions as outlined below.

**Editorial/Administrative**

1. Header updated with date and amendment #
2. Co-investigators removed from protocol cover page
3. Reformatted inclusion/exclusion criteria to link all criteria to the document structure
4. Corrected reference to inclusion criterion 4.1.14 cited in exclusion criterion 4.2.13.
5. Editorial clarifications made to inclusion/exclusion criteria for study
6. Section 6.2.1 typo corrected in table and 6.2.2 edits made to preparation of AP1903
7. Section 6.3 handling and disposal weblink per UNC IDS updated for fludarabine and cyclophosphamide
8. Adverse event, study management and regulatory sections of the protocol updated including sections 8.1.1, 8.1.2, 8.1.3, 8.3.3, 10.3, 10.4 and 10.8
9. Referenced NCI-CTCAE criteria 4.03 in 1.4.1, 3.1, 5.1.2, 7.2.2, 7.2.4, and sections 7.3.1-7.3.12
10. Updated section 12.2 Appendix B with latest version of management guidelines for CRS
11. Revised wording for collection of blood for correlatives in section 7.5
12. Removed exclusion criteria #4.2.7 and #4.2.8 excluding subjects with a history or known hypersensitivity to murine products

**Scientific**

13. The DLT/Safety assessment period was changed to 4 weeks to 6 weeks as indicated in the following sections: 1.1, 3.1, 3.3.2 5.1, 5.1.1, 5.1.2, 5.2.6, 5.2.8, Time and Events section 7.1 and footnotes #13, #15, 7.3.5, 7.3.6, 8.2, 8.4, 9.1, and Appendix 12.3

***THE ATTACHED VERSION DATED SEPTEMBER 25, 2017 INCORPORATES THE ABOVE REVISIONS***

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# PROTOCOL AMENDMENT #2

LCCC 1541-ATL: Administration of autologous CAR-T cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch in subjects with Acute Lymphoblastic Leukemia

**AMENDMENT INCORPORATES (check all that apply):**

X Editorial, administrative changes
X Scientific changes (IRB approval)
X Therapy changes (IRB approval)
X Eligibility Changes (IRB approval)

The protocol is amended to address comments provided by the FDA on January 13, 2017 and agreements reached during the follow up teleconference with the FDA held on January 17, 2017.

**Administrative / Editorial**

1. PI changed to Matthew Foster, MD, Philip Roehrs, MD is removed as PI and listed as a co-investigator. Other revisions made to coinvestigator list based on personnel changes
2. Revised the study synopsis section 1.1 to account for revisions to study design as Phase I dose escalation trial w/ dose determined in adults then children
3. Added text to section 1.4.1.1 on toxicity associated with CAR19 therapy to summarize current use and efficacy of tocilizumab
4. Revised section 1.5 Rationale for iC9-CAR19 T cells justification for Phase I dose escalation trial in section 1.5.1
5. Updated Appendix B CRS Toxicity Criteria grading to eliminate inclusion of co-morbidity index in adults and include management guidelines for CRS.
6. Appendices revised as now Appendix C Section 12.3 references Abbreviated follow up procedures and Appendix D Section 12.4 references Patient Reported Outcome assessments
7. Deleted references to Appendices that E and F that no longer apply to this version of the protocol in relevant sections of the protocol and ensured updated references to revised appendices throughout the text.
8. Deleted sub-study schema and sub-study description (section 5.2 in amend#1 version).
9. Deleted 5.2.1 (amend#1 version) that referenced definition of optimal dose of AP1903 for the sub-study evaluation.
10. To account for removal of the AP1903 dose finding sub-study, deleted all of the previous sub-study objectives (sections 2.3.1 and 2.3.2) and related sub-study endpoints (3.3)
11. Revised section 6.1.9 to remove impertinent SOP language
12. Section on CRS treatment with AP1903 related to dose finding portion of the trial was removed
13. Revised footnote #1 in section 7.1 Time and Events table to specify visit window for screening visits prior to procurement and lymphodepletion.
14. Clarified timing for safety reporting to start with procurement through the end of treatment in section 8.3.1
15. Revised section 8.3.3 Reporting requirements for pregnancy, FDA, RAC and IBC

16. Section 8.4 was updated to describe meetings and follow up procedures for patients participating in the dose escalation Phase I study. Sections related to meeting for the futility analysis and meetings around AP1903 dose finding were deleted
17. The Study management section of the protocol (section 10.0) was updated to reflect that this is a single institution study (sections 10.5.1, 10.5.2, and 10.6).
18. Added references to Appendix A Lansky or Karnofsky PS in study assessments sections 7.2 and 7.3
19. Revised section 7.7 bullet point #2 to eliminate wording related to re-infusion of cells.
20. Section 7.8 revised to note that assessment of safety will begin at the time of procurement.
21. Section 7.10.1 editorial correction made to bulleted points
22. Reference section updated to align with current version of the protocol

**Therapy**

23. Revised protocol does not allow for > 1 cell infusion of iC9-CAR19 T cells during dose escalation. Therefore, sections were deleted that allowed for administration of more than one dose of iC9-CAR19 T cells as follows:
    a. Previous section 12.4 Appendix D encompassing sections 12.4.1 - 12.4.9 including the time and events table and study-related assessments for a second or third cell infusion
    b. References to previous Appendix D deleted in section 5.1 and 5.3.6.
    c. Reference note on previous Appendix D in Section 7.1 deleted from footnotes
24. Study schema revised (section 5.1) and study description and treatment revised to reflect Phase I dose escalation in adults then children per 3+3 design. Footnotes to the study schema were also revised.
25. iC9-CAR-19 Treatment revised to account for different doses that may be evaluated during phase I study in section 5.2.3
26. Staggering of enrollment was added. The first subject must complete a 6 week safety follow-up period prior to additional subjects being enrolled on that dose level.
27. Section 6.2 was revised to reflect AP1903 dosing (0.4 mg/kg) in patients with G4 or G2/3 CRS that is not responsive to SOC

**Eligibility**

28. Inclusion criterion #2 revised to allow enrollment of patients 3-70 years of age.
29. Inclusion criterion #4 revised to more narrowly define eligibility of subiects with ALL that may participate in the trial.
30. Inclusion criterion #5 added to allow subjects with Ph+ ALL to enroll if they have failed ≥ 2 ABL tyrosine kinase inhibitors
31. Inclusion criterion #6 added requiring that lymphoblast CD19 expression is measured at the time of enrollment.
32. Inclusion criterion #9 renal and liver function test requirements for study entry changed to serum creatinine ≤1.5 x ULN, AST/ALT ≤ 3 x ULN, and Direct bilirubin ≤1.5 x ULN
33. Inclusion criterion #10 – editorial change noting pregnancy test should be completed 72 hrs prior to lymphodepletion
34. Exclusion criterion #2 revised to exclude patients with CNS3 disease, subjects with concurrent CNS3 disease and bone marrow relapse who have responded to CNS-directed therapy prior to enrollment will be allowed to participate. Subjects with CNS2 disease and

concurrent bone marrow relapse will be eligible. Intrathecal chemotherapy will be allowed to continue between lymphodepleting chemotherapy and cell infusion.

35. Revised exclusion criterion #13 to note that anti-CD19 antibody-based therapy OR cytotoxic chemotherapy not described as maintenance therapy in inclusion #12 within 2 weeks of procurement (not lymphodepletion).

**Scientific**

36. Updated the primary objective to indicate the subjects will be treated on more than 1 dose level.
37. Revised secondary objectives (section 2.2) to align with Phase I dose escalation trial in section 2.0
38. Revised secondary (section 3.2) endpoints to align with phase I dose escalation study objectives
39. Added an exploratory objectives/endpoints (Section 2.3 and 3.3) to account for allowance of AP1903 administration to patients with Grade 4 CRS or Grade 2-3 CRS unresponsive to SOC
40. Added an exploratory objective/endpoint (sections 2.3.2/3.3.2) to explore whether changes observed on a brief cognitive assessment are associated with the occurrence of CRS or neurotoxicity from iC9-CAR19 T cells
41. Reference criteria for grading CRS symptom severity based on publication of Lee et al. provided in Appendix B that now also includes management guidelines for CRS.
42. Appendix C – Comorbidity Index deleted / The determination of comorbidity index also deleted from time and events table (Section 7.1), TandE footnote, and pre-study assessment visit (section 7.2.2. Reference to comorbidity index deleted in sections relevant to sub-study which no longer applies)
43. Section 5.1.1 Dose escalation rules for 3+3 design added
44. Section 5.1.2 Dose limiting toxicities (DLTs) defined for cell infusion and deleted DLTs for AP1903 dose finding
45. Section 5.1.3 Sample collection plan pre/post AP1903 revised to account for single dose given (0.4 mg/kg)
46. Monitoring (Section 5.2.6) of patients during the 6-week evaluation period following the cell infusion: overnight inpatient observation (at least 12 hours) after cell infusion is now required. Fever will mandate hospitalization for subjects discharged after this hospitalization. We now require all phase I subjects to reside in local housing during the 6 week safety evaluation period after the cell infusion.
47. Details on cytokine monitoring and the cytokines being collection were added to section 7.1 footnote #14 as well as to relevant study assessment visits to coincide with the table of assessments for cytokine collection. The types of cytokines being evaluated were added to section 7.5
48. Section 8.1.4 was updated to include grade 4 or 5 infusion-related, CRS or neurotoxicity events as SAEs that should be reported expeditiously
49. Section 8.2 was revised to initiate safety monitoring at the time of procurement through the end of the safety assessment period which concludes 6 weeks after the cell infusion.
50. Added Mini-mental status exam (MMSE) to Time and Events table in section 7.1 and relevant assessment visit sections 7.2.2 -7.3.6
51. Added Appendix E – MMSE

LINEBERGER COMPREHENSIVE CANCER CENTER
CLINICAL ONCOLOGY RESEARCH PROGRAM
UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

***THE ATTACHED VERSION DATED May 17, 2017 INCORPORATES THE ABOVE REVISIONS***

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**PROTOCOL AMENDMENT #1**

LCCC 1541-ATL: Administration of autologous CAR-T cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch in subjects with Acute Lymphoblastic Leukemia

**AMENDMENT INCORPORATES (check all that apply):**

X Editorial, administrative changes
X Scientific changes (IRB approval)
X Therapy changes (IRB approval)
_ Eligibility Changes (IRB approval)

**Amendment Summary**

1. Minor editorial edit made to footnote of the main study schema in 5.1 and 2.3.2.4.
2. Addition of Co-Investigators Callie Coombs, MD; Christopher Dittus, DO; Brandi Reeves, MD; Satish Gopal, MD; Simon Tuchman, MD; Grace Park, PA; Alicia Pinto, ANP; Ashley Zanter, ANP; Megan McElfresh, PA-C; Angela Spruill, ANP
3. Minor editorial change to replace standard of care for CRS with institutional guidelines for CRS where applicable in the protocol
4. Clarified in Section 12.4 Appendix D Time and Events table for subsequent infusions + corresponding footnote 18 that subjects may be retreated with AP1903 at the same dose they received following the 1st infusion of ATLs, OR treated per institutional guidelines for CRS if the AP1903 dose they received previously is known to be ineffective at resolving CRS symptoms based on emerging data from the study
5. Clarified definition of worsening symptoms for CRS that allows subjects in the sub-study to receive additional supportive care for CRS section 5.2.
6. Removed language in Section 7.5.2 about IND reporting requirements that are not appropriate for the protocol
7. Added Patient Reported Outcomes Assessments measured by PROMIS Global Health and Physical Function questionnaires and selected items from the NCI PRO-CTCAE (see new sections 7.6 and Appendix F). Completion of the PRO questionnaires will be optional and only offered to adult study participants as outlined in the Time and Events tables in section Time and Events Tables in sections 7.1 and 12.4 (Appendix D). Footnotes to these tables were added to account for the PRO questionnaire assessments. Study Assessments in sections 7.2-7.3 and in 12.4.1-12.4.8 were updated to include these PRO questionnaire assessments. The addition of these assessments has been added to the study description in Section 5.1
8. Objective and Endpoint added to account for collection of QOL questionnaires to the main study section 2.2.6 and 3.2.6.
9. Subjects who experience disease progression after receiving a cell infusion(s) will still be required to complete abbreviated follow up procedures outlined in Appendix E (Section 12.5). Language added to sections 5.3.8 and 5.3.10 also clarify this point about abbreviated follow up requirements in subjects who experience disease progression.

10. Clarified when blood should be collected for HAMA testing prior to and following a second or third cell infusion (see section 12.4 (Appendix D) Time and Events Table footnote 13 and section 12.4.1.

11. Lymphodepleting chemotherapy will be given prior to the first cell infusion. All subjects will receive the same lymphodepleting regimen of fludarabine 25 mg/m$^2$/day administered i.v. over 30 min for three consecutive days and a single i.v. dose of cyclophosphamide 900 mg/m$^2$ administered over 1 hr on the fourth day. A single dose of cyclophosphamide (3g/m$^2$) administered over two hours has been removed as the lymphodepleting regimen specified for adults only (Sections 1.5.2, 5.1, 5.3, 6.3 and 7.2.3 were modified to include this change).

12. Mesna administration information removed as it is not required with 900 mg/m$^2$ dose of cyclophosphamide.

13. Noted that supportive care guidelines for administration of chemotherapy should be followed per UNC IDS policy in Section 5.3.7.

***THE ATTACHED VERSION DATED NOVEMBER 09, 2016 INCORPORATES THE ABOVE REVISIONS***

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# LCCC 1541-ATL: Administration of autologous CAR-T cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch in subjects with Acute Lymphoblastic Leukemia

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**IND #** 17295
**Version**: September 25, 2017

**LCCC1541-ATL**: Administration of autologous CAR-T cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch in subjects with Acute Lymphoblastic Leukemia

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**Signature Page**

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

**Principal Investigator (PI) Name**:_____

**PI Signature: _____**

**Date:_____**

**Version**: September 25, 2017

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# 1.0 BACKGROUND AND RATIONALE

## 1.1 Study Synopsis

LCCC1541-ATL is a Phase I dose finding trial to determine if chimeric antigen receptor T (CAR-T) cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch can be safely administered to adult and pediatric subjects with relapsed or refractory CD19+ acute lymphoblastic leukemia (ALL). The safety of iC9-CAR19 cells will be investigated in adult subjects initially before these same doses are tested in pediatric subjects using the 3+3 design. At least 6 subjects will be assigned in the adult dose-escalation portion of the study and followed for at least 4 weeks after the cell infusion before dose exploration in pediatric subjects can begin based on the 3+3 design. Dose finding in adult subjects will proceed independently from enrollment of dose finding cohorts of pediatric subjects. The starting dose of $5 \times 10^5$ transduced cells/kg (dose level 1) will enroll at least 3 adult subjects in the initial cohort. If there are no dose limiting toxicities (DLTs) within 4 weeks of the cell infusion in these 3 subjects, then the next cohort will evaluate $1 \times 10^6$ transduced cells/kg in adults. If there is toxicity in 1/3 subjects in the initial cohort, the cohort will be expanded to enroll up to 6 adult subjects. If dose level 1 is not well tolerated, de-escalation would occur to dose level -1 where subjects would receive $1 \times 10^5$ transduced cells/kg. During iC9-CAR19 T cell dose exploration, AP1903 (0.4 mg/kg), a dimerizing agent that is designed to engage and activate the caspase 9 safety switch to trigger iC9-CAR19 T cell death by apoptosis and thus alleviate the symptoms of cytokine release syndrome (CRS) will be given to subjects who develop grade 4 CRS or grade 2 or 3 CRS that is unresponsive to standard of care interventions. After the recommended phase 2 dose of iC9-CAR19 T cells has been determined in adults, the protocol will be amended to include a dose finding study of AP1903 in which subjects with lower grades of CRS are treated with iC9-CAR19 T cells and given AP1903 in an attempt alleviate CRS. The dosing strategy for AP1903 will be based on accumulated experience from the phase I portion of the trial. The ultimate goal is to determine the optimal dose of AP1903 that can be administered to adult and pediatric subjects with CRS that maximizes the proportion of subjects who experience CRS symptom resolution to $\leq$ Grade 1, while simultaneously preserving the anti-leukemia response mediated by autologous iC9-CAR19 T cell therapy.

## 1.2 Disease Background

Acute lymphoblastic leukemia (ALL) is a rare heterogeneous hematological malignancy in which precursor lymphoblasts fail to mature and proliferate rapidly in the bone marrow to supplant normal hematopoietic cells [1]. ALL is either B- or T-lymphoblastic leukemia with further classification of B-ALL into distinct entities defined by specific chromosomal abnormalities [2]. B-ALL is broadly categorized into Philadelphia chromosome-negative (Ph-neg) ALL, accounting for 75% of adult ALL cases, and Philadelphia chromosome-positive (Ph-pos) ALL which accounts for approximately 25% of adult ALL cases. Overall, ALL is the most common malignancy in childhood and represents approximately 25% of cancer diagnoses among children younger than 15 years of age. Its incidence decreases in adulthood (<1% of adult cancers) and gradually rises again among adults 50 years or older.

Most children (80% to 90%) are cured with conventional chemotherapies, however the same level of success has not been achieved in adults [3-5]. Although complete remission rates exceeding 90% are achieved after chemotherapy, the cure rate in adults with ALL is only 20-40%. The treatment of pediatric subjects with relapsed ALL remains a vexing problem as suboptimal re-induction remission rates coupled with poor long-term overall survival (OS) rates ranging from 15% to 50% require attention [6]. Effective agents are lacking in the relapsed/refractory setting in both adults and children and novel therapies are clearly warranted for these subjects.

## 1.3 Current Standard of Care

Frontline induction therapy for ALL often involves multi-agent chemotherapy consisting of an anthracycline, a vinca alkaloid, cyclophosphamide, and a corticosteroid [2]. Etoposide, methotrexate (MTX), cytarabine (Ara-C), and asparaginase are also commonly incorporated into frontline regimens. In addition, central nervous system (CNS) prophylaxis (or therapy) in the form of intrathecal (IT) or intraventricular MTX and/or Ara-C is an important component of frontline therapy for ALL. After attainment of morphologic complete remission (CR), which occurs in the majority of subjects (adults and children) with ALL, consolidation/intensification followed by maintenance therapy is a common practice, unless allogeneic hematopoietic stem cell transplant (allo-SCT) is an option in appropriate high-risk subjects. Despite the relatively high CR rates associated with frontline therapy, the 5-year survival among adolescents and adults collectively is approximately 40% [7], as opposed to 94% among the pediatric population [8]. The outcome discrepancy between adults and children may be attributable to differences in disease biology, treatment approach and tolerance to therapy [2]. The persistence of minimal residual disease (MRD), possibly represented by quiescent leukemia initiating cells that are resistant to conventional cytotoxic chemotherapy might explain the high rate of relapse among adults. This has prompted clinicians to incorporate MRD assessment at various time points after induction therapy in subjects with ALL.

### 1.3.1 Children with Relapsed/Refractory ALL

Treatment for relapsed ALL in children involves many of the same traditional backbone chemotherapy regimens initially used in frontline as well as hematopoietic stem cell transplantation (HSCT) [6]. Successful treatment of relapsed ALL has been closely correlated with length of the first clinical remission (CR1) and the site of relapse as outlined in Table 1 [9]. In general, children with a high risk for relapse or with intermediate risk and high MRD levels receive multi-drug chemotherapy followed by hematopoietic stem cell transplant (HSCT) [10]. Conversely, children with low or intermediate risks for relapse receive about 2 years of chemotherapy. Re-induction therapy comprises multiple blocks (3 to 9) of intensive chemotherapy.

**Table 1. Risk groups by duration of CR1 and site of relapse.**

| Risk group | CR1 duration and Site |
|---|---|
| Low | ≥18 months isolated extramedullary (Late IEM) |
| Intermediate | ≥36 months marrow (Late marrow),<br>< 18 months isolated extramedullary (Early IEM) |
| High | < 36 months marrow (Early marrow), |

| | |
|---|---|
| | all T-ALL/T-LL relapses |

IEM= isolated extramedullary

Salvage regimens may include the addition of novel agents (e.g. epratuzumab or bortezomib) added to the 3-block backbone of chemotherapy (Table 2) established by the Children's Oncology Group (COG) AALL01P2 trial. The primary goal is CR2 at the end of Block 1. However, the unfortunate reality is no substantial progress has been made in developing effective chemotherapy regimens for relapsed ALL in the past two decades and the chance for remission and cure is reduced with every subsequent relapse.

**Table 2. Backbone chemotherapy for relapse B-ALL**

| Block 1 | Block 2 | Block 3 |
|---|---|---|
| Pred 40mg/m$^2$/day, Days 1-29<br>VCR 1.5mg/m$^2$ Days 1, 7, 15, 21<br>PEG 2500 IU/m$^2$, Days 2, 9, 16, 23<br>Doxo 60mg/m$^2$, Day 1<br>IT AraC Day 1<br>IT MTX Days 15, 29 (or ITT if CNS positive) | ETOP Days 1-5<br>CPM Days 1-5<br>HDMTX Day 22 with leucovorin rescue | HD AraC Days 1, 2, 8, 9<br>L-Asp Days 2, 9<br>IT MTX or ITT Days 1, 22 |

Pred = prednisone; VCR = vincristine; PEG = pegylated L-asparaginase; IT = intrathecal; MTX = methotrexate; CNS = central nervous system; ITT = triple intrathecal therapy for CNS 3 only; ETOP = etoposide; CPM = cyclophosphamide; HD MTX= High dose methotrexate; HD AraC = High dose cytarabine

### 1.3.2 Adults with Relapsed/Refractory ALL

When making decisions about salvage therapy in adults several criteria require assessment, including full immunophenotyping, genotyping, and consideration of prior therapies, prior duration of remission, evaluation of sanctuary sites (especially CNS) and transplant candidacy. For relapsed or refractory ALL, salvage therapy may result in CR rates as low as 20% with median duration of response of 7 months, median OS of 3 months, and a 5-year OS of 7% [2]. Currently available salvage options include combination chemotherapy, clofarabine, an alternative tyrosine kinase inhibitor for Ph-pos ALL, nelarabine for T-ALL, vincristine sulfate liposome injection for Ph-neg ALL, and now blinatumomab for relapsed/refractory, Ph-neg, precursor B-ALL in adults. Certain young (i.e. <40 years of age) and fit subjects, subjects without CD19-expression, and those with long (>1-2 years) duration of initial remissions may benefit from multi-agent chemotherapy. Young adults may achieve responses with a pediatric salvage regimen evaluated in the COG study CCG-1941 [11]. Older subjects, multiple relapsed subjects, and those without targetable immunophenotypes can be treated with cytotoxic monotherapies. Subjects with relapsed precursor T-cell ALL have shown reasonable response rates with nelarabine (41% ORR). Both liposomal vincristine (35% ORR) and clofarabine (ORR 17%) have single agent activity in the relapsed setting as well, though response rates are low and the short durability of these remissions means that these therapies are palliative at best.

Recently, cellular therapies (i.e., chimeric antigen receptor; CAR-T cells) have demonstrated the highest remission rates and durability in relapsed/refractory ALL (see section 1.4) [12]. Due to the limited number of institutions capable of administering this therapy, and the need for treatment delays, such therapy is only suitable for subjects with

non-fulminant relapses. Blinatumomab is a novel agent that is a bispecific single chain antibody that targets CD19 antigen and redirects CD3+ T cells for selective lysis of tumor cells [2]. Blinatumomab has shown encouraging activity (43% CR/CRi) in subjects with CD19-expressing relapses and CD19+ MRD recurrences (76% MRD clearance) [13]. Blinatumomab is administered by continuous IV infusion (CIVI) for 4 weeks of a 6-week cycle. Because of the favorable activity and the toxicity profile of these targeted therapies, CD19 expression testing (flow cytometry or immunohistochemistry (IHC)) and consideration of immunotherapy is a preferred approach when possible. Unfortunately, blinatumomab has its limitations including a short half-life that necessitates long-term CIVI, a relapse-free response rate that is short-lived (i.e., 6 months) and the eventual emergence of CD19-negative clones [2].

### 1.4 Investigational Treatment – CD19 CAR-T therapy for ALL

CD19 is a 95-kDa B lineage-specific transmembrane glycoprotein, which functions as a central response regulator in B-lymphocytes. CD19 is expressed on B-cells during all stages of their differentiation and is maintained on cells that have undergone neoplastic transformation [14]. It is expressed on >95% of cells in subjects with B-ALL and recent studies have also shown that CD19 expression is maintained despite loss of CD20 expression following treatment with anti-CD20 antibodies. As a result, CD19 has become a promising target for therapies directed against B-cell malignancies including ALL. In fact, the most investigated target for chimeric antigen T cell receptor (CAR) therapy to date is CD19 [15].

Since B-cell leukemias and lymphomas are sensitive to cellular immune responses and antibody treatment, there has been a great deal of interest in combining both approaches through the generation of CARs. The introduction of CARs into T cells allows the rapid generation of effector cells specific for virtually any surface molecule [16], such as CD19 and has significantly increased the applicability of adoptive transfer of tumor-specific cytotoxic T lymphocytes (CTLs). CARs are generated by joining the heavy and light chain variable regions of a monoclonal antibody with a linker to form a single-chain Fv (scFv) molecule that is then fused with co-stimulatory endodomains (such as CD28 or 4-1BB) and the cytoplasmic portion of the T cell receptor's (TCR) ζ chain [16-19]. T lymphocytes engrafted with these molecules acquire the capacity to recognize and lyse tumor cells expressing the antigen recognized by the scFv and to proliferate in response to the antigen due to the activation of either CD28 or 4-1BB signaling pathways [19, 20].

The advantages of CARs over the native antibodies or ligands from which they are derived are a consequence of their physical association with effector T cells.[16, 21, 22] Thus, CAR-modified T cells can have an active biodistribution, with migration through multiple tissue planes along chemokine gradients, and can recruit the multiple cytotoxic effector mechanisms available to a T cell, rather than the more restricted cytotoxic machinery associated with, for example, the Fc component of an antibody. CARs also offer advantages over transfer of native alpha, beta T cell receptors (αβTCRs) of T lymphocytes. Target cell recognition by αβTCRs is major histocompatibility complex (MHC) restricted, precluding the design of a "universal" receptor for the treatment of subjects with different human lymphocyte antigen (HLA) polymorphisms. By contrast, CARs, like Mabs, are

essentially universal as their cytotoxic activity is MHC-unrestricted.[16] Moreover, many tumors down-regulate expression of MHC molecules and/or have dysfunctional antigen processing machinery, so that the target antigenic epitopes for αβTCR are simply not present. Since CAR-modified T cells bind directly to native proteins expressed on the surface of target cells without the need for antigen processing or MHC- restricted presentation, they are unaffected by this immune evasion strategy.

### 1.4.1 Clinical Experience with CD-19 CAR-T cells

CD-19 was initially chosen as a target of choice for CAR-based therapy because of its common expression in most B cell leukemias and lymphomas and its absence in normal tissues other than the B cell lineage [15]. Although the first clinical reports on CD19 CAR focused on non-Hodgkin lymphoma [23, 24] and chronic lymphocytic leukemia, [25, 26] the most impressive results have been obtained in ALL with response rates ranging from 50-90%. [25, 27-29] Maude et al., [29] recently published their experience with CD19 CAR in 30 subjects, half of whom had relapsed after allogeneic stem cell transplant. Ninety percent of subjects achieved a complete response at 1 month post-infusion, and 22 of 28 evaluable cases achieved MRD negative status with 67% event free survival rate at 6 months. Fifteen subjects received no further therapy post infusion, and durable responses appeared to correlate with higher peak levels of circulating CAR cells, as well as duration of B-cell aplasia. Cytokine release syndrome (CRS) developed to some extent in all subjects, with 8 (27%) subjects requiring admission to the intensive care unit for organ support. CRS was treatable with tocilizumab, a monoclonal antibody that blocks the IL-6 receptor, in 9 subjects with 6 subjects also requiring steroids.

Lee at al., [30] reported treating 21 subjects (age range = 1-30) with B-cell malignancies, 19 of which received the “prescribed” dose of CAR19 T-cells in a phase 1 dose escalation trial. Twelve of 20 subjects with ALL achieved MRD negative complete response with overall survival of 51.6% at 9.7 months and beyond. Leukemia free survival of these 12 subjects was 78.8% at 4.8 months, with all 10 subjects who went on to allo-SCT remaining disease free with no unexpected peri-transplant toxicities. CRS of any grade occurred in 16 subjects with 6 subjects (28.5%) experiencing grade 3 or greater toxicity. CRS was fully reversible in all subjects and managed with supportive care alone in 12 subjects, supportive care plus tocilizumab in 2 subjects, and supportive care plus tocilizumab and corticosteroids in 2 subjects. One subject experienced cardiac arrest after 4 days of CRS with successful resuscitation.

Overall, in concluded and ongoing CAR19 T cell ALL trials conducted in adult or pediatric subjects, responses with CAR19 T cell therapy has been impressive with complete remission rates as high as 90%. In larger studies now published by 3 groups [29-31] using different CD19 CAR designs, CR rates in subjects with ALL were in the 70% to 90% range [12]. Even more encouraging are the durable remissions observed in some subjects without additional therapy. All 3 ALL studies included subjects with a prior history of allogeneic-SCT and no graft vs host (GVHD) disease was seen.

#### 1.4.1.1 Toxicity of CAR19 T cells

Although CAR19 T cell therapy is efficacious, significant toxicities are seen in a proportion of subjects. Toxicities associated with CAR19 T cell therapy include hematologic toxicities introduced by required lympho-depleting regimens. In addition, CNS toxicities such as progressive confusion, aphasia, word finding difficulty, obtunded states with airway compromise and encephalopathy have been described [12]. Other toxicities observed with CAR19 T cell therapy include macrophage activation syndrome, CRS, sepsis, and B cell aplasia [15, 30-32]. To date, groups have not been able to predict nor control the duration of normal B-cell aplasia, and immunoglobulin infusions are required to reduce the risk of infection following CAR19 T cell therapy. Along with these noted toxicities, electrolyte disturbances and liver enzyme abnormalities have also been described.

Of the toxicities noted above, CRS is the most common toxicity experienced with CAR immunotherapy [33]. Symptoms can vary from mild flu-like symptoms to more severe toxicities such as vascular leak, hypotension, coagulopathy, pulmonary edema and multi-organ failure. CRS-associated toxicities, when severe, require intensive medical management including vasoactive pressors, mechanical ventilation, anti-epileptics, and antipyretics [33]. Cytokine elevations are detectable in most subjects, however the degree of elevation does not correlate with the severity of CRS or response to therapy. Similarly, the severity of CRS seems to be associated with the burden of leukemia at the time of therapy rather than the likelihood of remission [12]. The most significantly elevated cytokines associated with CRS are IL-10, IL-6, and IFN-γ. IL-6 is an inflammatory cytokine involved in a large number of immune processes, including neutrophil trafficking, acute phase response, angiogenesis, B cell differentiation, and auto-antibody production. In severe CRS with T-cell engaging therapies, IL-6 levels peak during maximal T cell proliferation. High levels of IL-6, present in the context of CRS likely initiates a pro-inflammatory IL-6-mediated signaling cascade.

The management of CRS to date involves supportive care measures and targeted therapy with tocilizumab (monoclonal antibody that blocks the IL-6 receptor) administered with or without corticosteroid therapy to suppress the immune response. Paradoxically, glucocorticoid administration, although effective in controlling toxicity, has led to decreased efficacy of CAR therapy as observed in the original report by Maude et al [12]. As IL-6 has been implicated in CRS, recent trials have demonstrated that tocilizumab therapy can attenuate CRS-related toxicity; however, no long term data exist regarding the impact of CRS treatment on CAR19 persistence and efficacy, and multiple subjects have severe toxicities that still require glucocorticoid administration. In view of these challenges, a treatment algorithm has been proposed for the management of CRS-associated toxicities (Figure 1) [33]. The goal of management is not to extinguish all evidence of CRS but to prevent life-threatening toxicity while maximizing the potential for antitumor effects.

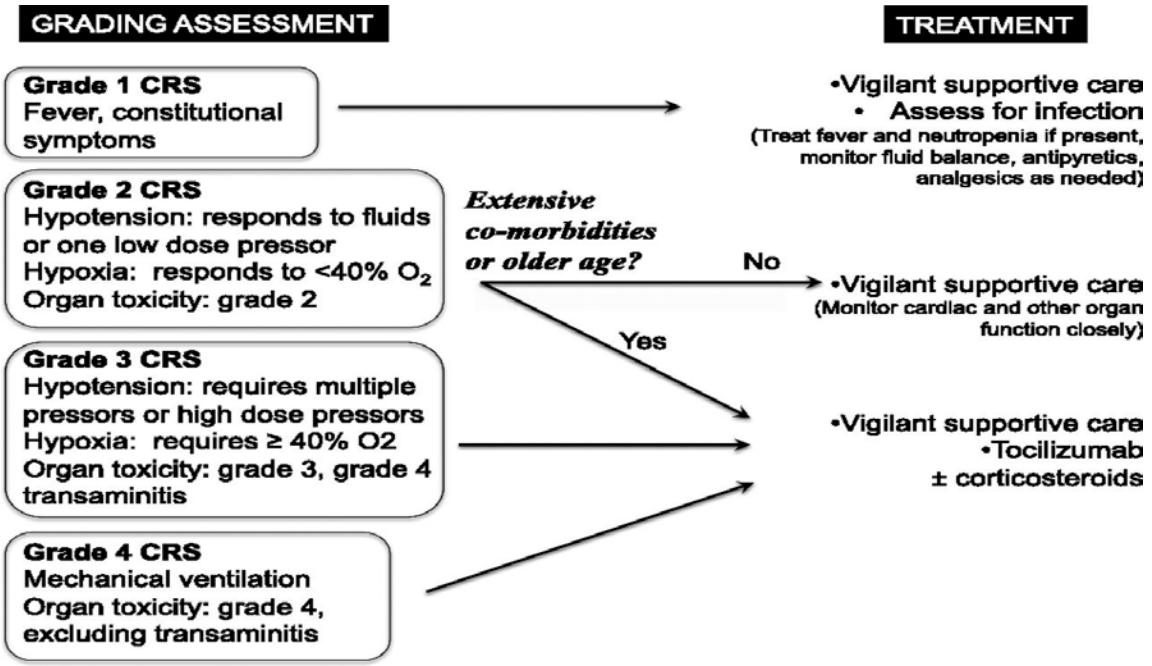



**Figure 1**. Treatment algorithm for management of CRS based on the revised CRS grading system. Vigilant supportive care including empiric treatment of concurrent bacterial infections and maintenance of adequate hydration and blood pressure for every grade. Immunosuppression should be used in all subjects with grade 3 or 4 CRS and instituted earlier in subjects with extensive comorbidities or older age. Grades 2-4 organ toxicities are dictated by CTCAE v4.03.

Trials are currently underway to determine the optimal timing for intervention with anti-cytokine therapy [34]. Current management guidelines delay administration of tocilizumab until after subjects develop more severe signs and symptoms of CRS because targeting IL-6 too early could inhibit CAR T-cell efficacy. This is based on the hypothesis that IL-6 may represent part of a cytokine feedback loop that enhances T-cell proliferation. Another hypothesis is that targeting IL-6, which is most likely released by macrophages, can manage CRS toxicity with less impact on CAR T-cell efficacy than corticosteroids. It has been observed that patients with CRS have marked elevations in ferritin, C-reactive protein (CRP) and soluble IL-2 receptor, findings consistent with macrophage activation syndrome or hemophagocytic lymphohistiocytosis [35]. Currently, the use of tocilizumab appears to be effective in most patients with limited inherent toxicity. In some cases, concomitant administration of corticosteroids with tocilizumab may be required to alleviate symptoms related to CRS [34, 35], though the use of corticosteroids has been associated with abrogation of CAR T-cell expansion and persistence [31]. For this reason, strategies to blunt severe CRS without compromising the therapeutic effect of CAR T-cells are needed.

## 1.5 Rationale for Therapy With T cells With an Inducible Caspase 9 Safety Switch

### 1.5.1 Inducible Caspase 9 Safety Switch

One alternative possibility to control the side effects due to CAR19 T cells and CAR T cells in general is based on the concept of the co-expression within CAR T cells of a safety switch gene that, when specifically activated, can rapidly eliminate the CAR T cells. We have recently clinically validated a novel safety switch that is based on the inducible human caspase 9 (iCasp9) [36, 37]. The iCasp9 construct consists of the sequence of the human FK506-binding protein (FKBP12) with an F36V mutation, connected through a Ser-Gly-Gly-Gly-Ser linker to the gene encoding human caspase 9, which is deleted of its endogenous caspase activation and recruitment domain. FKBP12-F36V binds with high affinity to an otherwise bioinert small molecule dimerizing agent, AP1903. In the presence of the drug, the iCasp9 pro-molecule dimerizes and activates the intrinsic apoptotic pathway, leading to cell death. Recently, the iCasp9 gene was used to genetically modify donor derived T lymphocytes that were then infused into subjects receiving a haploidentical stem cell transplant to promote faster immune reconstitution [37]. The modified cells could expand and function *in vivo*; in those subjects who developed GVHD after infusion of iCasp9 T lymphocytes, a single dose of AP1903 (0.4 mg/kg) activated the safety switch and eliminated more than 90% of the injected T cells within 30 minutes of drug administration, permanently reversing GVHD.

Since the inclusion of iCasp9 in donor-derived T cells allows for their rapid elimination when these cells cause GVHD in the context of haploidentical transplant, iCasp9 seems a valuable safety switch to include in CAR19 T cells to rapidly and permanently remove them if severe CRS develops (**Figure 2**).

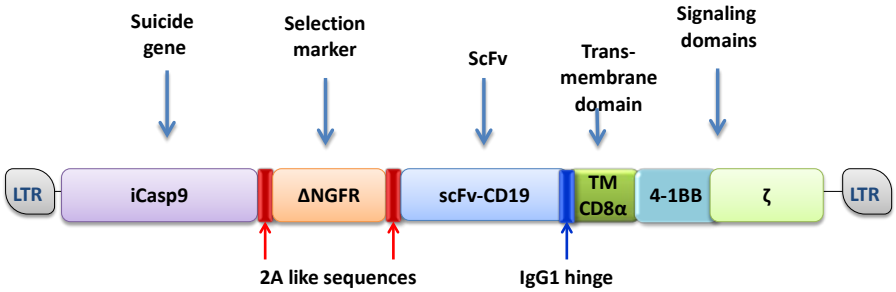



**Figure 2. Structure of the iC9.2A.ΔNGFR.2A.CAR.CD19 (iC9-CAR19) vector** comprising the iCasp9 sequence, the truncated low-affinity nerve growth factor receptor (ΔNGFR) serving as the selectable marker and the CAR19. The sequence cassette is then incorporated into the SFG retroviral vector. In transduced cells, the selection with NGFR allows the enrichment in CAR19+ cells and the administration of AP1903 leads to dimerization of iCasp9 resulting in a caspase cascade that ends in apoptosis resulting in the rapid elimination of CAR19 T cells.

Unfortunately, like corticosteroids or other genetic safety switches, elimination of CAR19 T cells by activation of iCasp9 triggered by the infusion of AP1903 will also diminish the therapeutic effect of CAR19 T cells. However, our preclinical experiments suggest that unlike corticosteroids and other safety switches, the function of this iCasp9 safety switch can be pharmacologically-controlled with administration of AP1903 leading to a more fine-tuned elimination of CAR19 T cells in instances where CRS-associated toxicity may be

serious or life-threatening. Importantly, this pharmacologically-controlled safety switch allows for the persistence of CAR19 T cells in the peripheral blood and thus may preserve antitumor efficacy compared to current approaches for the treatment and management of CRS (manuscript in preparation).

AP1903 which activates the iCasp9 gene has been evaluated in a phase 1 dose escalation safety study conducted in healthy volunteers [38]. No significant adverse effects were noted when AP1903 was administered over a 0.01 mg/kg to 1.0 mg/kg dose range via intravenous (i.v.) infusion over 2 hours. The only drug-related adverse event observed at any of the dose levels tested was facial flushing (vasodilation) in one volunteer at the 1.0 mg/kg dose level. The drug exhibited a rapid distribution phase with mean $C_{max}$ values ranging from 10 to 1275 ng/ml over the 0.01 to 1.0 mg/kg dose range. Following infusion, plasma levels were reduced to 18%, 7% and 1 % of the maximal concentration at 0.5, 2, and 10 hrs post dose. In our recent study, subjects who developed GVHD after infusion of iC9-CAR19 T cells were treated with 0.4 mg/kg of AP1903, as a 2 hour i.v. infusion [37]. We confirmed results from previously published studies of AP1903 using an in vitro biological assay that showed no detectable levels of drug in the plasma within 24-48 hours of administration.

Based on this evidence, we hypothesize that T lymphocytes collected from subjects and genetically modified to co-express the iCasp9 gene will expand *in vivo* upon infusion into subjects and furthermore, exhibit anti-leukemic activity. To test this hypothesis, we plan to infuse autologous activated T cells transduced with a gamma-retroviral vector encoding iCasp9 and CAR19 into adult and pediatric subjects with relapsed/refractory CD19+ ALL. We will conduct a phase I dose escalation study to determine a tolerable dose of iC9-CAR19 T cells that can be administered. The safety of iC9-CAR19 cells will be investigated in adult subjects initially before these same doses are tested in pediatric subjects using the 3+3 design. The doses we plan on testing are cell doses that have been investigated in trials of other CD19-directed CAR-T cell constructs [29, 30, 39]. We anticipate that should CRS develop following the infusion of iC9-CAR19 cells, the in vivo expansion of these cells can be rapidly controlled and downregulated by the administration of AP1903 via its interaction with iCasp9.

### 1.5.2 Rationale for Conditioning Chemotherapy

Conditioning chemotherapy (e.g., lymphodepleting chemotherapy) has been shown to improve the persistence of modified T cells and has been associated with improved progression free survival.

Brentjens et al also studied the safety and persistence of chimeric antigen receptor T cells with antibody against CD19 and found improved clinical benefit in subjects who received prior conditioning chemotherapy and had low tumor burden or minimal residual disease [25]. In addition, subjects that were treated with modified T cells without prior cyclophosphamide had very low frequency of T cells detected in the peripheral blood. In contrast, subjects received cyclophosphamide conditioning had T cells more readily detected over time in the blood and bone marrow.

Conditioning chemotherapy reduces the patient's tumor burden which seems to enhance the persistence of the adoptively transferred T cells [40]. An inverse relationship between the persistence of CAR T cells and the peripheral blood tumor burden was also demonstrated in subjects with metastatic neuroblastoma [41].

Lymphodepleting chemotherapy also reduces the number of the host's suppressive cells, such as regulatory T cells, allowing the genetically modified T cells to expand and eradicate tumor cells [40, 42]. The conditioning regimen may also stimulate the production of cytokines such as IL-7 or IL-15 which may also favor expansion of the infused cells [17, 43]. Grupp et al showed improved T-cell counts and proliferation in subjects who received CAR-T cells on day 2 after conditioning chemotherapy compared to day 90, supporting the theory that T cell infusion should occur early after conditioning [44].

In this trial, adult and pediatric subjects will receive lymphodepleting chemotherapy prior to infusion of iC9-CAR19 cells. Both adult and pediatric subjects will receive a lymphodepleting regimen of fludarabine 25 mg/m$^2$/day administered i.v. over 30 min for three consecutive days and a single i.v. dose of cyclophosphamide 900 mg/m$^2$ administered over 1 hr on the fourth day. This lymphodepleting regimen has been safely administered to pediatric subjects in a previous CAR-T therapy trial [30].

### 1.6 Correlative Studies

Correlative studies are outlined in section 7.5 of the protocol.

## 2.0 STUDY OBJECTIVES

### 2.1 Primary Objective

To determine the safety and tolerability of autologous iC9-CAR19 T cells administered to adult and pediatric subjects with relapsed or refractory CD19+ ALL.

### 2.2 Secondary Objectives

**2.2.1** To identity a recommended phase 2 dose (RP2D) of iC9-CAR19 T cells in adult and pediatric subjects with relapsed or refractory CD19+ ALL.

**2.2.2** To measure the survival of iC9-CAR19 T cells in vivo.

**2.2.3** To determine the anti-leukemia overall response rate (ORR) mediated by autologous iC9-CAR19 T cells administered to adult and pediatric subjects with relapsed or refractory CD19+ ALL.

**2.2.4** To determine overall survival (OS) in adult and pediatric subjects with relapsed or refractory CD19+ ALL following infusion of iC9-CAR19 T cells.

**2.2.5** To determine event-free survival (EFS) in adult and pediatric subjects with relapsed or refractory CD19+ ALL following infusion of iC9-CAR19 T cells.

**2.2.6** To determine relapse-free survival (RFS) in adult and pediatric subjects with relapsed or refractory CD19+ ALL following infusion of iC9-CAR19 T cells.

**2.2.7** To measure patient-reported symptom, physical function, and health-related quality of life at baseline and over time in subjects treated with iC9-CAR19 T cells.

### 2.3 Exploratory objectives

**2.3.1** To determine the utility of the safety switch in iC9-CAR19 T cells by allowing for administration of AP1903 (0.4 mg/kg dose) to subjects with grade 4 CRS or grade 2 or 3 CRS that is unresponsive to standard of care interventions (ie, does not resolve to grade 0-1 within 24 hours after 2 doses of tocilizumab, with sum of doses ≥12mg/kg) following infusion of iC9-CAR19 cells to adult and pediatric subjects with relapsed or refractory CD19+ ALL.

**2.3.2** To explore whether changes observed on a brief cognitive assessment are associated with the occurrence of CRS or neurotoxicity from iC9-CAR19 T cells.

## 3.0 Endpoints

### 3.1 Primary Endpoint

Toxicity will be classified and graded according to the National Cancer Institute's Common Terminology Criteria for Adverse Events (CTCAE, version 4.03) and CRS symptoms will be graded according to criteria outlined in Section 12.2 Appendix B: CRS Grading Criteria and Management Guidelines.

(**Note**: The safety evaluation period for DLT assessment will encompass toxicities related to the cell therapy that are experienced starting on the day of iC9-CAR19 T cell infusion up through 4 weeks post infusion. See section 5.1.2 of the protocol for definitions of DLTs and RP2D. General safety monitoring will begin at the time of procurement).

### 3.2 Secondary Endpoints

**3.2.1** The recommended phase 2 dose of iC9-CAR19 cells will be determined based on 3+3 dose finding rules as specified in section 5.1.1 and the tolerability of iC9-

CAR19 cells assessed by NCI-CTCAE criteria and CRS grading criteria outlined in Section 12.2 Appendix B: CRS Grading Criteria and Management Guidelines.

**3.2.2** Persistence of iC9-CAR19 T cells in vivo will be determined by quantitative polymerase chain reaction (PCR) and flow cytometry in samples of peripheral blood.

**3.2.3** ORR (CR/CRi) to iC9-CAR19 T cell therapy will be determined using National Comprehensive Cancer Network Response Criteria (NCCN) for ALL provided in Section 7.9.1. Assessment of MRD will be included as criterion of response (ie, the percentage of subjects who achieve CRm defined as MRD negative CR by either flow cytometry or PCR analysis will be determined).

**3.2.4** OS will be measured from the date of administration of iC9-CAR19 T cells to the date of death.

**3.2.5** EFS applies to all subjects and will be measured from the date of administration of iC9-CAR19 T cells to the date of signs and symptoms of treatment failure or relapse from CR or CRi, or death from any cause; subjects not known to have any of these events are censored on the date they were last examined.

**3.2.6** RFS will apply only to subjects achieving CR or CRi and measured from the date of achievement of a remission until the date of relapse or death from any cause; subjects not known to have relapsed or died at last follow-up are censored on the date they were last examined.

**3.2.7** Patient reported symptoms will be measured using selected symptoms from the NCI PRO-CTCAE. Patient-reported physical function will be measured using the PROMIS Physical Function Score derived from the PROMIS Physical Function Short Form 20a v1.0. Patient-reported health-related quality of life will be measured using the PROMIS Global Health Score derived from the PROMIS Global Health Short Form v1.0-1.1. (see Appendix D: Patient Reported Outcome Surveys).

## 3.3 Exploratory Endpoints

**3.3.1** The efficacy of AP1903 (0.4 mg/kg) will be assessed by pre to post dose diminutions in iC9-CAR19 cell viability in vivo, cytokine production, and CRS symptom grade to 0-1 per criteria provided in Section 12.2 Appendix B: CRS Grading Criteria and Management Guidelines.

**3.3.2** Cognitive function will be assessed by the Mini-Mental Status Examination (MMSE, score 0-30, see section 12.5 Appendix E: Mini-Mental State Exam) pre- and post- infusion of iC9-CAR19 T cells during the 4-week safety evaluation period after cell infusion.

# 4.0 SUBJECT ELIGIBILITY

All clinical and laboratory data required for determining eligibility must be available in the subject's medical/research record which will serve as the source document. Subjects may be transfused with blood products to obtain a hemoglobin level >8.0 g/dL and platelet count > 20,000 per µl.

**Note**: During the period after cell procurement and during iC9-CAR19 T-cell production, subjects are allowed to receive standard of care therapy for ALL to manage their disease if the treating physician feels it is in the subject's best interest.

## 4.1 Inclusion Criteria

Subjects must fulfill all of the following inclusion criteria to participate in this study:

**4.1.1** Written informed consents signed by subject or legal guardian of a pediatric subject and HIPAA authorization for release of personal health information. NOTE: HIPAA authorization may be included in the informed consent or obtained separately. Subjects must sign a consent to undergo cell procurement. Written informed consent to enroll in the iC9-CAR19 cell therapy trial must be obtained prior to lymphodepletion.

**4.1.2** Age 3 to 17 years of age for pediatric subjects (weight must be ≥ 10 kg), ≥ 18 to 70 years of age for adults at the time of consent.

**4.1.3** Karnofsky score > 60% if > 10 years old or Lansky performance score of greater than 60% if ≤ 10 years old (See Appendix A: Performance Status (Lansky and Karnofsky).

**4.1.4** Relapsed or refractory precursor B cell ALL:

- Second or greater bone marrow relapse OR
- Any bone marrow relapse >100 days after allogeneic stem cell transplant OR
- Primary refractory ALL defined as no complete response after 2 cycles of a standard of care chemotherapy regimen OR
- For adult subjects: first bone marrow relapse with duration of first CR <1 year OR CR1 duration >/=1 year and refractory to >/= 1 cycle of therapy for treatment of relapse
- For pediatric subjects: first bone marrow relapse refractory to 1 cycle of standard therapy for relapsed ALL
- While active CNS3 leukemia will be excluded, subjects with concurrent CNS3 disease and bone marrow relapse who have responded to CNS-directed therapy prior to enrollment will be allowed to participate. Intrathecal chemotherapy will be allowed to continue between lymphodepleting chemotherapy and cell infusion.
- Subjects with CNS2 disease and concurrent bone marrow relapse will be eligible. Intrathecal chemotherapy will be allowed to continue between lymphodepleting chemotherapy and cell infusion

**4.1.5** Subjects with Ph+ ALL will be eligible if they have failed ≥ 2 ABL tyrosine kinase inhibitors. Subjects with the T315I ABL kinase point mutation will be eligible if they have failed ponatinib-containing therapy, regardless of the number of prior ABL tyrosine kinase inhibitors.

**4.1.6** CD19 positivity of lymphoblasts confirmed by flow cytometry or IHC per institutional standards.

**4.1.7** Life expectancy ≥ 12 weeks.

**4.1.8** Subjects who have received prior therapy with murine antibodies must have documentation of absence of human anti-mouse antibodies (HAMA) prior to enrollment/lymphodepletion on this study.

**4.1.9** Demonstrate adequate renal and hepatic function as defined in the table below; all screening labs to be obtained within 72 hrs prior to procurement, lymphodepletion and cell infusion. See inclusion 4.1.15 below for other requirements prior to lymphodepletion and cell infusion.

| **System** | **Laboratory Value** |
|---|---|
| **Renal** | |
| Serum Creatinine | ≤ 1.5 x ULN |
| **Hepatic** | |
| Bilirubin | ≤ 1.5 x upper limit of normal (ULN), unless attributed to Gilbert's Syndrome |
| Aspartate aminotransferase (AST) | ≤ 3.0 × ULN |
| Alanine aminotransferase (ALT) | ≤ 3.0 × ULN |

**4.1.10** Females of childbearing potential must have a negative serum pregnancy test within 72 hours prior to procurement and again 72 hours prior to lymphodepletion. NOTE: Females are considered of child bearing potential unless they are surgically sterile (have undergone a hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or they are naturally postmenopausal for at least 12 consecutive months.

**4.1.11** Females and males of childbearing potential must be willing to abstain from heterosexual activity or to use 2 forms of effective methods of contraception from the time of informed consent until 3 months after treatment discontinuation. The two contraception methods can be comprised of two barrier methods, or a barrier method plus a hormonal method. Female participants will inform their male partners that they must use the methods of birth control required by the protocol.

**4.1.12** Male subjects with female partners must have had a prior vasectomy or agree to use an adequate method of contraception (i.e., double barrier method: condom plus spermicidal

agent) starting with the first dose of study therapy through 3 months after the last dose of study therapy.

**4.1.13** As determined by the enrolling physician or protocol designee, ability of the subject to understand and comply with study procedures.

**4.1.14** Subjects currently receiving "maintenance" doses of chemotherapy are eligible and the need for intrathecal prophylaxis prior to procurement or lymphodepletion is left to the discretion of the investigator. Maintenance doses of chemotherapy are defined as methotrexate ≤30 mg/m$^2$/week, mercaptopurine ≤100 mg/m$^2$/day and vincristine ≤ 2 mg/28 days. Corticosteroid-containing maintenance therapy is permitted only if corticosteroids are administered >14 days prior to procurement. (Note: Corticosteriod use with doses at the discretion of the treating physician are allowed after procurement up to the beginning of lymphodepletion. Corticosteroid use is contraindicated following iC9-CAR19 infusion unless medically necessary e.g., to treat CRS).

**4.1.15** To Qualify for Autologous T Lymphoctyte (ATL) Cell Infusion:

- Subjects must have autologous transduced activated T-cells with ≥ 80% expression of CAR19 (must be confirmed prior to lymphodepletion)
- Bilirubin ≤1.5 times the upper limit of normal (ULN)
- AST ≤ 3 times ULN
- Serum creatinine ≤1.5 times ULN
- Pulse oximetry of > 90% on room air

## 4.2 Exclusion Criteria

Subjects meeting any of the following criteria CANNOT be enrolled in this study:

**4.2.1** Subjects with relapsed fulminant CD19+ ALL that is rapidly progressing who cannot safely delay definitive treatment for their ALL by at least 4 weeks in the opinion of the investigator.

**4.2.2** Lumbar puncture must be performed prior to enrollment and subjects with evidence of CNS3 disease will be excluded from study entry. Subjects with concurrent CNS3 disease and bone marrow relapse who have responded to CNS-directed therapy prior to enrollment/lymphodepletion will be allowed to participate. Subjects with CNS2 disease

and concurrent bone marrow relapse will be eligible. Intrathecal chemotherapy will be allowed to continue between lymphodepleting chemotherapy and cell infusion.

**4.2.3** Pregnant or breastfeeding (NOTE: breast milk cannot be stored for future use if the milk is collected while the mother is being treated on study).

**4.2.4** Has a known additional malignancy that is active and/or progressive requiring treatment; exceptions include basal cell or squamous cell skin cancer, in situ cervical or bladder cancer, or other cancer for which the subject has been disease-free for at least five years.

**4.2.5** Subjects must not have tumor in a location where enlargement could cause airway obstruction.

**4.2.6** Subjects may not have an oxygen requirement as defined by pulse oximetry of < 90% on room air.

**4.2.7** Treatment with any investigational drug within 21 days (ie, three weeks) prior to ATL infusion or has received any tumor vaccines within the previous six weeks prior to ATL infusion.

**4.2.8** Subjects must not have left ventricular ejection fraction of <40% (shortening fraction <27% for pediatric subjects) as measured by echocardiogram or MUGA.

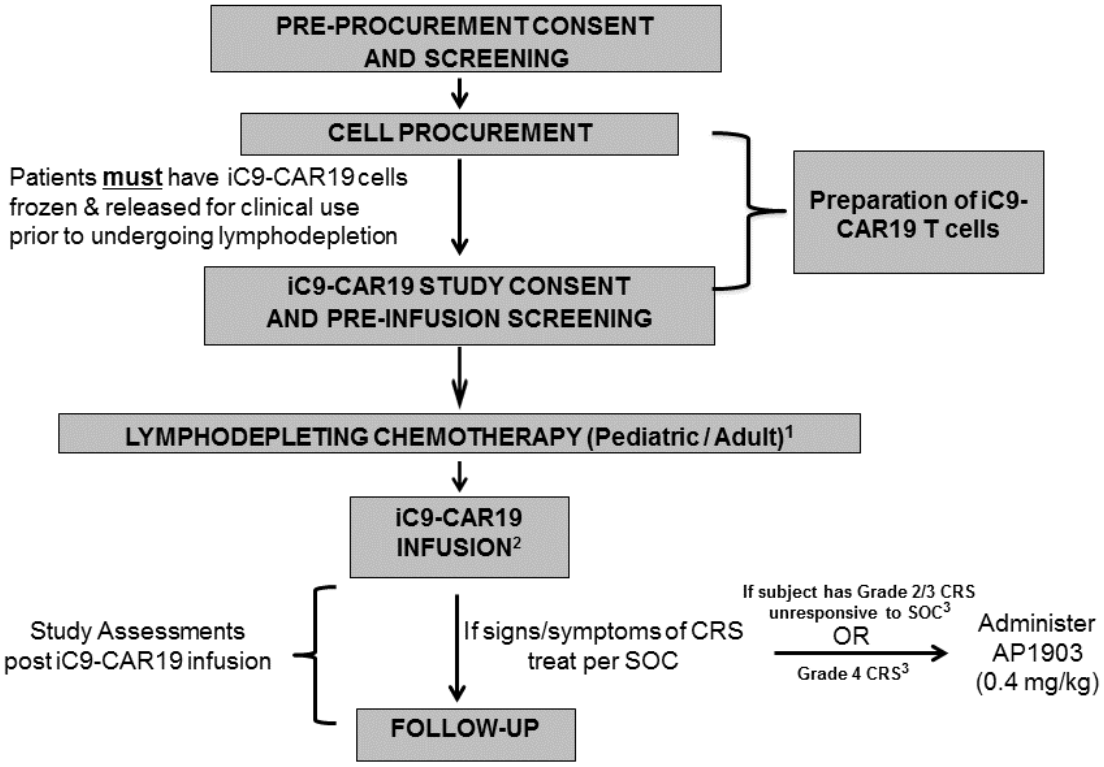
**4.2.9** Active infection (fungal, bacterial or viral) including HIV, HTLV, HBV, HCV (tests can be pending at the time of cell procurement; only those samples confirming lack of active infection will be used to generate transduced cells) Note: To meet eligibility subjects are required to be negative for HIV antibody or HIV viral load, negative for HTLV1 and 2 antibody or PCR negative for HTLV1 and 2, negative for Hepatitis B surface antigen, or negative for HCV antibody or HCV viral load.

**4.2.10** Prior to procurement current use of systemic corticosteroids at doses ≥10mg/day prednisone or its equivalent; those receiving <10mg/day may be enrolled at discretion of investigator. (Note: Corticosteroid use with doses at the discretion of the treating physician are allowed after procurement up to the beginning of lymphodepletion. Corticosteroid use is contraindicated following iC9-CAR19 infusion unless medically necessary e.g., to treat CRS).

**4.2.11** Received anti-CD19 antibody-based therapy OR cytotoxic chemotherapy not described as maintenance therapy in inclusion 4.1.14 within 2 weeks of procurement.

# 5.0 TREATMENT PLAN

## 5.1 Study Schema




[1]All subjects (adults and children) will be given fludarabine 25 mg/m$^2$ x 3d, cyclophosphamide 900 mg/m$^2$ on d4 (See sections 5.2.2 and 6.3 for details).
[2]iC9-CAR19 T cell infusion to occur 2-4 days after lymphodepletion. Escalating doses of iC9-CAR19 cells tested in adults and then children using 3+3 rules. See sections 5.1.1 Dose Escalation Rules (3+3) and section 5.2.3 Administration of iC9-CAR19 T cells for additional information.
[3]Subjects with grade 4 CRS or grade 2/3 CRS unresponsive to standard of care (SOC) interventions (ie, does not resolve to grade 0-1 within 24 hours after 2 doses of tocilizumab, with sum of doses ≥12mg/kg) will be given 0.4 mg/kg of AP1903.

This single center, open-label Phase I dose finding trial seeks to determine if CAR-T cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch can be safely administered to adult and pediatric subjects with relapsed or refractory CD19+ ALL. The safety of iC9-CAR19 cells will be investigated initially in adult subjects before these same doses are tested in pediatric subjects using the 3+3 design (see section 5.1.1). At least 6 subjects will be assigned in the adult dose-escalation portion of the study and followed for at least 4 weeks after the cell infusion before dose exploration in pediatric subjects can begin. Dose finding in adult subjects will proceed independently from enrollment of dose finding cohorts in pediatric subjects. The starting dose of 5 x $10^5$ transduced cells/kg (dose

level 1) will enroll at least 3 adult subjects in the initial cohort. If there are no DLTs within 4 weeks of the cell infusion in these 3 subjects, then the next cohort will evaluate 1 x $10^6$ transduced cells/kg in adults. If there is toxicity in 1/3 subjects in the initial cohort, the cohort will be expanded to enroll 3 more subjects. If dose level 1 is determined to be above a tolerable dose, de-escalation would occur to dose level -1 where subjects would receive 1 x $10^5$ transduced cells/kg. The first subject enrolled on each dose level will be evaluated for a 4 week DLT safety follow-up period prior to enrolling additional subjects on that dose level.

During dose exploration, AP1903 (0.4 mg/kg), a dimerizing agent that is designed to engage and activate the caspase 9 safety switch to trigger iC9-CAR19 T cell death by apoptosis and thus alleviate the symptoms of CRS will be given to subjects who develop grade 4 CRS or grade 2 or 3 CRS that is unresponsive to standard of care interventions. Once the recommended phase 2 dose of iC9-CAR19 T cells has been determined in adults, the protocol will be amended to include a dose finding study of AP1903 in which subjects are treated with iC9-CAR19 T cells and given AP1903 for CRS. The dosing strategy for AP1903 will be based on accumulated experience from the phase I portion of the trial at the time of the amendment.

**Dose levels of iC9-CAR19 cells to be tested in adult and pediatric subjects:**

| Dose level | Dose (#transduced cells/kg) | Maximum dose for BMI >35 (#transduced cells) |
|---|---|---|
| -1 | 1 x $10^5$ | 1.25 x $10^7$ |
| 1 | 5 x $10^5$ | 6.25 x $10^7$ |
| 2 | 1 x $10^6$ | 5 x $10^8$ |

**Cell procurement, lymphodepletion, cell infusion, and safety and disease assessment procedures for all subjects**: Peripheral blood cells (PBSCs) will be collected for creation of iC9-CAR19 cells from consenting subjects who meet eligibility for cell procurement (See Section 5.2.1) prior to their collection for this purpose. Approximately four weeks later, pediatric or adult subjects for whom cells have been successfully generated and who meet eligibility criteria for lymphodepletion will undergo lymphodepleting chemotherapy. Pediatric and adult subjects will receive lymphodepleting chemotherapy consisting of i.v. fludarabine 25 mg/$m^2$/day administered for 3 consecutive days followed by i.v. cyclophosphamide at 900 mg/$m^2$ on the $4^{th}$ day. Subjects who meet eligibility criteria for cellular therapy will receive iC9-CAR19 T cells within 2-4 days after the lymphodepleting regimen followed by close monitoring over the ensuing 4 weeks with disease assessments scheduled at 4 and 8 weeks after the cell infusion.

**Quality of life assessments (optional and for adults only):** Patient reported outcomes (PRO) will be measured by PROMIS Global Health and Physical Function questionnaires and selected items from the NCI PRO-CTCAE (see section 12.3 Appendix D: Patient Reported Outcome Surveys). Completion of the PRO questionnaires will be optional and only offered to adult study participants as outlined in the Time and Events tables in section Time and Events Table in sections 7.1.

### 5.1.1 Dose Escalation Rules (3+3)

| Number of Patients with DLT | Action |
|---|---|
| 0 out of 3 subjects | Escalate to next dose level |
| 1 out of 3 subjects | Accrue 3 additional evaluable patients at current dose level |
| 1 out of 6 subjects | Escalate to next dose level |
| 2 or more subjects in a dosing cohort | TCD has been exceeded. Expand a prior cohort tested at a lower dose level unless 6 patients have been assigned to that dose already |

Tolerable cell dose = A TCD is defined as the dose at which approximately 0.20 of patients experience DLT (0 – 1 out of 6 patients).

- Dose escalation of iC-CAR19 cells will follow 3+3 dose escalation rules in adult and pediatric subjects

- The exploration of iC9-CAR19 cell doses will begin in adults first. At least 6 adult subjects must be treated with iC9-CAR19 cells before dose exploration can begin in pediatric subjects

- The dose cohorts for adults will be filled independently from the dose escalation cohorts of pediatric patients

- The first subject evaluated in each dose cohort must complete the 4-week evaluation period before a second subject is dosed in that cohort. Additionally, prior to escalating or de-escalating to the next dose level, all subjects in the prior cohort must have cleared the 4-week DLT evaluation window.

- The doses of iC9-CAR19 T cells to be explored are provided in section 5.2.3

- While the RP2D of iC9 CAR19 T cells may be the TCD, the determination of RP2D will be made after taking into account TCD as well as other factors such as iC9 CAR19 T cell persistence, expansion and cell manufacturing variables.

### 5.1.2 Definition of Dose limiting toxicity

An event will be considered a DLT per NCI CTCAE criteria v 4.03 or CRS grade criteria outlined in section 12.2 Appendix B: CRS Grading Criteria and Management Guidelines of the protocol if it occurs within the DLT reporting period (ie, 4 week evaluation period following iC9-CAR19 cell infusion) as specified below:.

- Any treatment-emergent Grade 3- 4 CRS event that does not decrease to Grade $\leq 2$ within 7 days
- Any treatment-emergent NCI-CTCAE Grade 5 event
- Any treatment-emergent autoimmune toxicity $\geq$ Grade 3.
- NCI-CTCAE Grade 3-5 allergic reactions related to the study cell infusion.

- NCI-CTCAE Grades 3 and greater organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) not pre-existing or due to the underlying malignancy and occurring within 30 days of study product infusion.
- Treatment-emergent NCI-CTCAE hematologic Grade 4 toxicity that is not attributed to residual leukemia and does not resolve within 21 days.

DLTs are defined as at least as possibly related to iC9-CAR19 T cells. If an apparent DLT is clearly due underlying leukemia and is unrelated to the cell infusion, then the investigator will specify that the event is not a DLT.

### 5.1.3 Sample Collection Plan Pre/Post AP1903 Administration

Subjects who develop grade 4 CRS or grade 2 or 3 CRS that is unresponsive to standard of care interventions (ie, does not resolve to grade 0-1 within 24 hours after 2 doses of tocilizumab, with sum of doses ≥12mg/kg) will be given AP1903 (0.4 mg/kg). Blood samples will be collected at various time points before and after AP1903 to assess CRP, ferritin, cytokine levels and iC9-CAR19 T cell viability *in vivo* as outlined in the table below.

Apart from these additional samples to be collected in subjects treated with AP1903 (0.4 mg/kg) who develop grade 4 CRS or grade 2 or 3 CRS that is unresponsive to standard therapy, all subjects in the study should be followed as outlined in the Time and Events Table in section 7.0.

| **Time point relative to infusion of AP1903** | **Correlative studies** | **CRP/Ferritin** |
|---|---|---|
| Pre-dose (w/in 30 min prior to infusion) | X[1] | X[2,] |
| 2 hrs after the end of infusion | X[1] | |
| 24 hrs after the end of infusion | X[1,3] | X[2] |

1. Collect 2 mL of blood at the specified times noted above pre/post AP1903 dose (0.4 mg/kg given as a 2-hr i.v. infusion).
2. Collect 3 mL of blood pre-dose (w/in 30 min prior to first infusion AP1903) for measurement of CRP and ferritin.
3. Collect an additional 10 mL of blood for correlative studies (see section 7.5.5)

Samples leftover from cytokine and cell viability testing will be stored for future analysis of AP1903 if needed.

## 5.2 Treatment Plan

### 5.2.1 Cell Procurement

Peripheral blood, up to 300 mL total (in up to 3 collections) will be obtained from subjects for cell procurement. In subjects with low (CD3 count as assayed by flow cytometry less

than 200/μl) T-cell count in the peripheral blood, a leukopheresis may be performed to isolate sufficient T cells. The parameters for pheresis will be 2 blood volumes.

### 5.2.2 Lymphodepleting regimen

Subjects will receive a lymphodepleting regimen of fludarabine 25 mg/m$^2$/day administered i.v. over 30 min for three consecutive days and a single i.v. dose of cyclophosphamide 900 mg/m$^2$ administered over 1 hr on the fourth day.

### 5.2.3 Administration of iC9-CAR19 T cells

Post lymphodepletion, subjects who meet eligibility criteria for cellular therapy will receive iC9-CAR19 T cells within 2-4 days after completing the pre-conditioning chemotherapy regimen. We will administer iC9-CAR19 post lymphodepletion at dose levels specified in the table below. A phase I trial performed by Lee et al. established that $1\times10^6$ CAR19+ T cells/kg was safe and associated with significant in vivo expansion and we anticipate similar results with iC9-CAR19+ T cells [30].

**Dose levels of iC9-CAR19 cells to be tested during dose escalation:**

| Dose level | Dose (#transduced cells/kg) | Maximum dose for BMI >35 (#transduced cells) |
|---|---|---|
| -1 | $1 \times 10^5$ | $1.25 \times 10^7$ |
| 1 | $5 \times 10^5$ | $6.25 \times 10^7$ |
| 2 | $1 \times 10^6$ | $5 \times 10^8$ |

### 5.2.4 Premedication

Subjects may be pre-medicated with Benadryl (diphenhydramine) up to 1 mg/kg IV (max 50 mg) and Tylenol (acetaminophen) 10 mg/kg po (max 650 mg). We will pre-medicate subjects who have a history of reactions to blood products. Steroids should be avoided given their detrimental effect on the survival of the infused T cells. Anti-emetics in appropriate dosage for each subject will be prescribed as necessary.

### 5.2.5 Cell Administration

iC9-CAR19 T cells will be given by a licensed healthcare provider via intravenous injection over 5-10 minutes through either a peripheral or a central line. The volume of infusion will depend upon the concentration of the cells when frozen and the size of the subject. The expected volume will be 1-50cc.

### 5.2.6 Monitoring of Subjects in the Study

All subjects will require overnight inpatient observation (at least 12 hours) after the cell infusion. Fever will mandate hospitalization for subjects discharged after this hospitalization. All phase I subjects must reside in local housing (as designated by the

Cellular Therapeutics Program Local Housing SOP) during the 4-week DLT/safety evaluation period. Subjects with medical indications for inpatient care or those whose physicians assess that outpatient care will pose an undue risk will remain inpatient for their treatment until indications for inpatient care have resolved.

Subjects will be monitored as outlined in the Time and Events Table in Section 7.0. Institutional guidelines will be followed for the monitoring and treatment of tumor lysis syndrome should the need arise.

### 5.2.7 Supportive Care

Subjects will receive supportive care for acute or chronic toxicity, including blood components or antibiotics, and other interventions as appropriate. Supportive care guidelines for administration of chemotherapy should be followed per UNC IDS policy.

### 5.2.8 Concomitant Medications/Treatments

Ideally, subjects should not receive other antineoplastic agents for at least 4 weeks post T cell infusion (for purposes of evaluation); however, subjects with progressive disease may receive other therapy if needed at the discretion of their attending physician. If subjects receive other therapy they will come off study for adverse event reporting after the initial 4-week assessments outlined in Section 7.1 are completed. These subjects will continue to be monitored after according to the Time and Events Table in Appendix C (Section 12.3).

If subjects experience progressive disease after the initial 4-week assessment period following the cell infusion has been completed, they should be followed according to the Time and Events Table in Appendix C (Section 12.3).

### 5.2.9 Duration of Therapy

Therapy in LCCC1541-ATL involves 1 infusion of iC9-CAR19 cells (see section 5.2.3). Treatment with one infusion will be administered unless:

- Subject decides to withdraw from study treatment, **OR**
- General or specific changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator.

### 5.2.10 Duration of Follow-Up

Subjects will be followed for up to 15 years for RCR evaluation or until death, whichever occurs first. Subjects removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

**Subjects who experience unequivocal disease progression after receiving a cell infusion will still be required to complete abbreviated follow up procedures outlined in Appendix C (Section 12.3)**.

### 5.2.11 Removal of Subjects from Protocol Therapy

Subjects will be removed from protocol therapy and the PI notified when any of the criteria listed in Section 7.10 apply. The reason for discontinuation of protocol therapy will be documented on the eCRF.

In the case where a subject decides to prematurely discontinue protocol therapy ("refuses treatment"), the subject should be asked if she or he may still be contacted for further scheduled study assessments. The outcome of that discussion should be documented in both the medical records and in the eCRF.

### 5.2.12 Study Withdrawal

If a subject decides to withdraw from the study (and not just from protocol therapy) all efforts should be made to complete and report study assessments as thoroughly as possible. The investigator should contact the subject or a responsible relative by telephone or through a personal visit to establish as completely as possible the reason for the study withdrawal. A complete final evaluation at the time of the subject's study withdrawal should be made with an explanation of why the subject is withdrawing from the study. If the reason for removal of a subject from the study is an adverse event, the principal specific event will be recorded on the eCRF. Excessive subject withdrawals from protocol therapy or from the study can render the study un-interpretable; therefore, unnecessary withdrawal of subjects should be avoided.

### 5.2.13 Cytokine Release Syndrome Therapy

Subjects who develop CRS should be treated for CRS according to standard of care guidelines outlined in Section 12.2 Appendix B: CRS Grading Criteria and Management Guidelines.

Subjects who develop grade 4 CRS or grade 2 or 3 CRS that is refractory to standard of care interventions (ie, does not resolve to grade 0-1 within 24 hours after 2 doses of tocilizumab, with sum of doses ≥12mg/kg) may be treated with AP1903 (0.4 mg/kg) i.v. dose administered over 2 hours.

# 6.0 STUDY TREATMENT INFORMATION

## 6.1 Autologous T Lymphocytes (ATL) iC9-CAR.19 CELLS

### 6.1.1 Construction of the iC9-CAR19 Vector

The single chain antibody (scFv) (FMC63) targeting the CD19 molecule was cloned in frame with the human IgG1hinge, the CD8a transmembrane domain, 4-1BB and ζ endodomains. The iCasp9 and ΔNGFR genes were previously described. The entire cassette (iC9-CAR19) was then cloned into the retroviral vector SFG (provided by R.C. Mulligan, Cambridge, Massachusetts) that is a Moloney murine leukemia (Mo-MuLV)

virus-based vector. A schematic representation of the retroviral construct is shown in **Figure 2 (Section 1.5.1).**

### 6.1.2 Generation of Transduced Cells

All manufacturing procedures (from 6.1.4 onwards) will be performed in our GMP facility as dictated by Standard Operating Protocols (SOP). Brief summaries are given here.

### 6.1.3 Source Material

See section 5.2.1 (procurement).

### 6.1.4 Activated T Lymphocytes

Activated T Lymphocytes will be generated using our previously validated SOP. Briefly, PBMC will be activated with anti-CD3 and anti-CD28 antibodies, and then supplemented with IL-7 and IL-15 [45]. Anti-CD3 and anti-CD28 are now available in GMP grade and our validation studies show improved cell transduction when both anti-CD3 and anti-CD28 antibodies are combined.

### 6.1.5 Retroviral Production

A retroviral producer line will be generated for the construct at UNC. A master cell-bank of producer cells will be generated and tested to exclude production of replication competent retrovirus and infection by Mycoplasma, HIV, HBV, HCV and others. Supernatant generated from the master-cell bank will be produced by a certified vendor. All batches of retroviral supernatant will be tested for sterility and to exclude RCR as issued with the CofA and as directed by the SOPs and the Vector Production master-file with the FDA. For this specific study we will use batches of retrovirus released for clinical use.

### 6.1.6 Transduction

ATL are transduced 3-4 days after initiation as described previously using recombinant Fibronectin fragment CH-296 (Retronectin$^{TM}$, Takara Shuzo, Otsu, Japan) coated plates or bags. Virus is attached to retronectin by incubating producer supernatant in coated plates or bags. Cells are then transferred to virus coated plates or bags.

### 6.1.7 *Ex Vivo* Expansion

After transduction, transgenic ATL will be expanded supplementing them with IL-7 and IL-15 twice a week. By day 8 - 10 ATL cells will be selected using anti-NGFR antibody and CliniMACS System Miltenyi Biotech to enrich in NGFR transduced cells. Selected ATL will be further expanded to reach sufficient numbers for adoptive transfer by feeding them with IL-7 and IL-15 twice a week. After transduction and selection a small number of cells will be removed to evaluate for transduction efficiency using flow cytometry.

### 6.1.8 Freezing

When sufficient number for cell infusion is achieved, cells will be collected and frozen following previously validated SOPs. Cells will be also tested for cytotoxicity against CD19+ tumor cells and CD19 negative cell lines to check receptor function and for sensitivity to the CID drug. All lines will be checked for identity, phenotype and

microbiological culture and cryopreserved prior to administration according to SOPs. The results will be reviewed by QA prior to issuing a Certificate of Analysis (CofA).

### 6.1.9 Testing

Products that meet study specific release criteria, as detailed on the CofA that accompany each infusion product, will be infused as per Section 5.2.3.

If a positive sterility testing result is reported after the product is infused, the Principal Investigator, UNC IRB, and FDA, and other relevant parties will be notified as per our manufacturing SOPs for deviation management and notification.

### 6.1.10 Potential Toxicities

Potential toxicities may be categorized as those related to infusion of T cells; transduction; cross-reactivity with normal tissues; cytokine release syndrome (CRS); and tumor lysis syndrome. In addition to the information presented below, also see section 1.4.1.

Retroviral Transduction

There has been concern that even a single retroviral integration can contribute to oncogenesis. Insertional upregulation of cellular proto-oncogenes was the main cause of 5 serious adverse events in two different X-chromosomal linked severe combined immunodeficiency (X-SCID) gene therapy trials. The French study reported 4 cases. Fischer's group replaced the missing common gamma chain (γc) in X-SCID subjects with ex- vivo retroviral transduction of autologous stem cells. Four of 11 treated subjects developed T-ALL associated with a single retroviral integrant [46, 47]. The integration site of the retrovirus was similar. More recently a case of leukemia has also been reported in a second study in SCID subjects conducted in UK. It is likely that correction of common-gamma chain deficiency and related immunodeficiency syndromes represent special cases. The γc is a shared component of IL-2, IL-4, IL-7, IL-9 and IL-15. Hence it is a crucial component of T- cell proliferation and thymogenesis. A proliferative advantage is expected for progeny of stem cells with functional γc. In female carriers of X-SCID there is a pattern of non-random X-inactivation in T-cells, B-cells and NK-cells [48]. Moreover a subject with X-SCID developed substantial numbers of T-cells following reversion of the mutant allele in a single hematopoietic stem cell [49]. High efficiency retroviral transduction of human stem cells is difficult to achieve even with GALV pseudotyping. It is likely that in X-SCID few truly pluripotent stem cells were transduced and the stem cell pool expressing the highest level of transgene (due to integration at a transcriptionally active site) then undergo numerous doublings to restore the entire T-cell compartment. Random mutations caused by this supra-physiological proliferation combined with retroviral integration in a transcriptionally active region led to leukemogenesis.

To date more than 200 subjects have received genetically modified cells in clinical trials including subjects we have treated on our protocols - using retrovirally marked autologous marrow [50] or retrovirally-marked EBV-CTLs [51-53]. In none of these has malignancy caused by retroviral transduction been reported. However, there is a possibility that the vector could randomly integrate into a site that could lead to leukemogenesis.

Second malignancies with standard therapy for ALL occur at rates of approximately 1% in children [54] and 1.43% in adults [55]. The rates of second malignancies for specific salvage chemotherapy regimens are less known, but given that such regimens employ DNA-damaging chemotherapy, these regimens likely add to the risks noted above. In light of this, the natural history and poor prognosis of relapsed ALL, and given the entire previous experience with retroviral gene therapy we feel that the risks of retroviral induced leukemogenesis are small and are justified in this subject group.

Cross-reactivity
CD19 is only expressed by B lymphocytes and thus the only toxic effect will be B cell aplasia which has been described in other trials using CAR.CD19-redirected T cells.

### 6.1.11 Concomitant Therapy with IVIG

Intravenous immunoglobulin (IVIG) will be administered to prevent infection in subjects with the expected occurrence of hypogammaglobulinemia per institutional standard practice.

### 6.1.12 Blood Draw Limits for Pediatric Patients

The total amount of blood drawn from each pediatric subject will not be more than 3 mL (< 1 teaspoon) per 2.2 lbs of the subject's weight.

## 6.2 AP1903

Subjects who develop Grade 4 CRS or grade 2 or 3 CRS that is unresponsive to standard of care interventions (ie, does not resolve to grade 0-1 within 24 hours after 2 doses of tocilizumab, with sum of doses ≥12mg/kg) will receive 0.4 mg/kg of AP1903 (See section 5.1.2 and Section 12.2 Appendix B: CRS Grading Criteria and Management Guidelines) as an i.v. infusion over 2 hrs.

### 6.2.1 Supplier/How Supplied

AP1903 for injection is formulated as a 5 mg/mL solution of AP1903 in a 25% solution of the non-ionic solubilizer Solutol HS 15 (250 mg/mL). At room temperature the formulation is a clear, colorless solution. Upon refrigeration, this formulation undergoes a reversible phase transition, resulting in a slightly opaque white solution. This phase transition is reversed upon rewarming to room temperature.

| Component | Function | Nominal amount per vial | Concentration |
|---|---|---|---|
| AP1903 | API | 10 mg | 5 mg/mL |
| Solutol HS 15 | Solubilizer | 20 gm | 250 mg/mL |

AP1903 for injection should be warmed to room temperature prior to dilution with normal saline.

AP1903 for injection is incompatible with materials containing plasticizer or DEHP and materials sterilized with ethylene oxide.

Bellicum Pharmaceuticals is the supplier of AP1903 and will be provided to subjects at no costs by the manufacturer. Clinical supplies will be affixed with a clinical label in accordance with regulatory requirements.

This trial is open-label; therefore, the subject, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

### 6.2.2 Instructions for Preparation and Infusion

AP1903 for Injection is a concentrated solution of 2.33 ml in a 3 ml vial, at a concentration of 5 mg/ml, (i.e., 10.66 mg per vial). Prior to administration, the calculated dose will be diluted to a final concentration of 0.4 mg/ml in 0.9% normal saline for infusion.

AP1903 will be administered via IV infusion over 2 hours, using a non-DEHP, non-ethylene oxide sterilized infusion set and an infusion pump.

### 6.2.3 Handling and Dispensing

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label.

Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site.

Clinical supplies may not be used for any purpose other than that stated in the protocol.

### 6.2.4 Recommendations for Administration

Premedication per institutional standards (acetaminophen and antihistamine) 30 minutes prior to infusion for prevention of potential hypersensitivity to the Solutol HS15 or AP1903 is recommended.

### 6.2.5 Stability and Storage

AP1903 for injection is stable for at least 24 months when stored at the recommended temperature of 2-8$^0$C.

### 6.2.6 Return and Retention of Study Drug

The investigator is responsible for keeping accurate records of the clinical supplies received from Bellicum Pharmaceuticals or designee, the amount dispensed to and returned by the subjects and the amount remaining at the conclusion of the trial.

Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy (e.g., UNC IDS drug destruction policy). It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

### 6.2.7 Adverse Events Associated with AP1903

Three Phase I clinical trials have been completed with AP1903. The first study was a single center, randomized placebo- and saline-controlled ascending single dose study in healthy volunteers (n=28) [38]. No significant adverse effects were noted when AP1903 was administered over a 0.01 mg/kg to 1.0 mg/kg dose range via intravenous (i.v.) infusion over 2 hours. The only drug-related adverse event observed at any of the dose levels tested was facial flushing (vasodilation) in one volunteer at the 1.0 mg/kg dose level. There were no treatment related changes in vital signs, ECG data, clinical laboratory data, bleeding time or platelet aggregation data in this study.

A second study referred to as the CASPALLO trial evaluated a single 0.4 mg/kg dose of AP1903 administered to subjects who had received ascending doses of iCaspase T cells [37]. Four subjects between the ages of 3 and 17 years who had undergone stem-cell transplantation for relapsed acute leukemia and developed GVHD were given a single i.v. dose (0.4 mg/kg) of the dimerizing drug AP1903. This dose eliminated more than 90% of the modified T cells within 30 minutes after administration and ended the GVHD response without recurrence. No drug-related adverse events were reported in this trial.

A phase I/II safety and efficacy study known as the Bellicum BPX101 trial was conducted in 18 male subjects with metastatic castration resistant prostate cancer who had received gene modified dendritic cells (Presented at the Annual Meeting of the American Society of Clinical Oncology in 2011; Abstract 4670). BPX-101 which targets prostrate specific membrane antigen was administered intra-dermally every 2 weeks X 6 doses, followed 24 hours later by an i.v. infusion of AP1903 (0.4 mg/kg). One subject exhibited cytokine reaction upon infusion of AP1903 as a result of the activation of modified dendritic cells and one subject experienced urticarial and flushing which resolved with antihistamine treatment. No other AP1903-related events were reported. In general, AP1903 has an unremarkable safety profile with minimal risks for toxicity. Other adverse events unrelated or of improbable relationship to the administration of AP1903 reported in studies include chest pain, flu syndrome, halitosis, headache, injection site pain, vasodilatation, increased cough, rhinitis, rash, gum hemorrhage and ecchymosis.

Please refer to the Investigators Brochure for additional information on AP1903.

## 6.3 Lymphodepletion

Subjects will receive a lymphodepleting regimen of fludarabine 25 mg/m$^2$/day given i.v. over 30 min for three consecutive days and a single i.v. dose of cyclophosphamide 900 mg/m$^2$ given over 1 hour on the fourth day.

**Fludarabine**

Indications: Fludarabine is indicated in the treatment of adult subjects with B-cell chronic lymphocytic leukemia who have not responded to or whose disease has progressed during treatment with at least one standard alkylating agent containing regimen.

Mechanism of action: Fludarabine phosphate is a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-β-D-arabinofuranosyladenine (ara-A). Fludarabine phosphate

is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite acts by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. Inhibition of DNA synthesis interferes with the expansion of rapidly proliferating malignant cells.

Product description: Fludarabine is supplied as 50 mg in a single-dose vial (1 vial per carton). Store vials at $20^0$ to $25^0$C ($68^0$F to $77^0$F); excursions permitted between $15^0$ to $30^0$C ($59^0$F to $86^0$F).

Solution preparation: Fludarabine phosphate for injection should be prepared for parenteral use by aseptically adding sterile water for injection, USP. When reconstituted with 2 mL of sterile water for injection, USP, the solid cake should fully dissolve in 15 seconds or less; each mL of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg, mannitol, and sodium hydroxide to adjust the pH to 7.7. In clinical studies, the product is diluted in 100 mL or 125 mL of 5% Dextrose Injection, USP or 0.9% Sodium Chloride, USP.

Dose and Route of administration: A single IV dose of fludarabine 25 mg/m$^2$ will be administered i.v. over 30 min for three consecutive days.

Possible side effects:

- Myelosuppression (neutropenia, thrombocytopenia and anemia)
- Fever and chills
- Fatigue and weakness
- Infection and pneumonia
- Nausea and vomiting and diarrhea
- Malaise, mucositis and anorexia
- Serious opportunistic infections such as latent viral reactivation, herpes zoster, Epstein-Barr virus, and progressive multifocal leukoencephalopathy

Full prescribing information on fludarabine is available at**:**
http://medlibrary.org/lib/rx/meds/fludarabine-1/

Handling and Disposal: Please see policy on hazardous drugs:
http://intranet.unchealthcare.org/intranet/hospitaldepartments/safetynet/policies/hazardousdrugs.pdf

Local requirements for disposal of hazardous drugs should be followed per institutional policy.

**Cyclophosphamide**
Indications: Cyclophosphamide is an alkylating agent indicated for treatment of malignant diseases including malignant lymphomas, Hodgkin's disease, lymphocytic lymphoma, mixed-cell type lymphoma, histiocytic lymphoma, Burkitt's lymphoma, multiple

myeloma, leukemias, mycosis fungiodes, neuroblastoma, adenocarcinoma of the ovary, retinoblastoma and breast carcinoma.

Mechanism of action: Cyclophosphamide is an alkylating agent that has anticancer activity. It is converted in the liver to active alkylating metabolites such as phosphoramide mustard by cytochrome-P450 enzymes. These alkylating metabolites interfere with the growth of susceptible rapidly proliferating malignant cells. Alkyl radicals intercalate into DNA strands and interfere with DNA replication.

Product description: Cyclophophosphamide is supplied as 500 mg, 1 gm and 2 gm vials containing white powder for IV administration. Store vials at or below $25^0$C ($77^0$F). Cyclophosphamide does not contain any antimicrobial preservative and care must be taken to assure the sterility of prepared solutions. USE ASEPTIC TECHNIQUE.

Solution preparation: Cyclophosphamide should be reconstituted in 25 mL (500 mg), 50 mL (1 gm) or 100 mL (2 gm) sterile water for injection, USP, for IV infusion. Shake vigorously to dissolve the drug. To minimize risk of dermal exposure, always wear gloves when handling vials containing cyclophosphamide sterile powder for injection.

Dose and Route of administration: A single IV dose of cyclophosphamide at 900 mg/m$^2$ will be administered over 1 hr on the 4$^{th}$ day of the lymphodepletion regimen in this study in pediatric subjects.

Possible side effects:

- Nausea, vomiting, diarrhea
- Urinary bladder toxicity
- Bone marrow suppression
- Gonadal suppression
- Myelodysplasia
- Alopecia
- Immunosuppression
- Hyperpigmentation of the skin

Full prescribing information on cyclophosphamide is available at:
http://medlibrary.org/lib/rx/meds/cyclophosphamide-1/

Handling and Disposal: Please see policy on hazardous drugs:
http://intranet.unchealthcare.org/intranet/hospitaldepartments/safetynet/policies/hazardousdrugs.pdf

Local requirements for disposal of hazardous drugs should be followed per institutional policy.

- Nausea
- Vomiting

# 7.0 EVALUATIONS AND ASSESSMENTS

## 7.1 Time and Events Table

| Study Assessments | Screening[1] | | | | ALTCAR Infusion[19] | Follow up (continued on next page) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-procurement | | Pre-lympho-depletion | | Wk 0 D1[1] | Wk 0 D3 & D5 | Wk 1 D1[1] | Wk 1 D3 & D5 | Wk 2 D1[1] | Wk 3 D1[1] | Wk 4 D1[1] | Wk 6 D1[1] | Wk 8 D1[1] |
| History | X | | X | | X | X | X | X | X | X | X | X | X |
| Physical exam[1] | X | | X | | X | X | X | X | X | X | X | X | X |
| Mini-mental status exam (adults only) | | | X | | X | X | X | X | X | X | X | | |
| Performance status | X | | X | | X | | | | X | | X | X | X |
| Pregnancy test[2] | X | | X | | | | | | | | | | |
| PFTs and EF[3] | X | | | | | | | | | | | | |
| Pulse oximetry[3] | X | | X | | X | X | X | X | X | X | X | | |
| CBC with diff and platelets[4] | X | | X | | X | X | X | X | X | X | X | X | X |
| Virus testing[5] | X[5] | | | | | | | | | | | | |
| Procurement of cells | After screening[6] | | | | | | | | | | | | |
| Complete Metabolic Panel (CMP) + additional clinical lab tests[7] | X | | X | | X | X | X | X | X | X | X | X | X |
| ALL Assessment[8] | X | | X | | | | | | | | X | | X |
| Immunoglobulin G (IgG); IgA at baseline only | | | X | | | | | | | | X | | X |
| Function & persistence tests[9] | | | X | | X[9] | | X[9] | | X | X[9] | X | X | X |
| RCR by PCR and archive samples[11] | | | X | | | | | | | | | | |
| Quantitative PCR[12] | | | X | | X[12] | | X[12] | | X | X | X | X | X |
| HAMA testing[13] | | | X[13] | | | | | | | | X[13] | | |
| Cytokine Testing + CRP + IL-2Rα | | | | | X[14] | | X | | X | X | X | X | X |
| Toxicity of cell infusion[15] | X | | X | | X | X | X | X | X | X | X[15] | | |
| CofA | | | X[16] | | | | | | | | | | |
| PRO Questionnaires (optional) [17] | X | | | | X | X[17] | X | X[17] | X | X | X | X | X |
| Infuse iC9-CAR19 cells | | | | | X[18] | | | | | | | | |
| AP1903 (For treating CRS after ATL infusion)[19] | | | | | **See Section 5.1.3** / Time points for blood sampling if AP1903 given for G4 CRS or G2/3 CRS refractory to SOC | | | | | | | | |
| Tissue studies | | | **See Section 7.5** | | | | | | | | | | |

LYMPHODEPLETION

### 7.1 Time and Events Table (continued)

| **Study Assessments** | **Follow up** | | | | | |
|---|---|---|---|---|---|---|
| | **Mth 3 D1[1]** | **Mth 6 D1[1]** | **Mth 9 D1[1]** | **Mth 12 D1[1]** | **Every 6 mths x 4 yrs[1]** | **Yearly[1]** |
| History | X | X | X | X | X | X |
| Physical exam[1] | X | X | X | X | X | X |
| Performance status | X | X | X | X | X | X |
| Pregnancy test[2] | | | | | | |
| PFTs and EF[3] | | | | | | |
| Pulse oximetry[3] | | | | | | |
| CBC with diff and platelets[4] | X | X | X | X | X | |
| Virus testing[5] | | | | | | |
| Procurement of cells | | | | | | |
| Complete Metabolic Panel (CMP) + additional clinical lab tests | X | X | X | X | | |
| ALL Assessment[8] | X | X | | X | See 7.3.12 | |
| Immunoglobulin G (IgG); IgA at baseline only | X | X | X | X | X | X |
| Function & persistence tests[9] | X | X | X | X | X[10] | X |
| RCR by PCR and archive samples[11] | X | X | | X | | X[11] |
| Quantitative PCR[12] | X | X | X | X | X[12] | X |
| HAMA testing[13] | | | | | | |
| Cytokine Testing + CRP + IL-2R-alpha | | | | | | |
| Toxicity of cell infusion | | | | | | |
| CofA | | | | | | |
| PRO Questionnaires (optional) [17] | X | X | X | X | X | X |
| Infuse iC9-CAR19 cells | | | | | | |
| Tissue studies | **See Section 7.5** | | | | | |

## Time and Events Table Footnotes

1. Screening includes tests to confirm eligibility within 14 days prior to procurement and 72 hours prior to lymphodepletion; Physical exam to include assessment of clinical status based on NCI-CTCAE criteria v4 starting on Day of autologous T lymphocyte (ATL) cell infusion and checked at each visit through follow up to capture adverse events related to clinical deterioration. **NOTE:** some pre-infusion screening tests may not be performed until the day of infusion. A window of +/-3 days will apply to all study visits including for the first 8 weeks unless otherwise noted. A window of +/- 24 hrs will apply to the visits scheduled during the first two weeks following the cell infusion (i.e., during week 0 and week 1). A window of +/-10 days will apply to the every 3 months study visits, and a window of +/-30 days will apply to visits separated by ≥ 6 months. Yearly follow-up visits are required during long-term follow-up for a total of 15 years. Annual physical exam only required for up to 5 years after the last cell infusion. Physical exam to include height (baseline only), weight and vital signs.

2. Serum pregnancy testing will be done in female subjects of childbearing potential within 72 hours prior to procurement and repeated 72 hours prior to lymphodepletion.

3. PFTs = pulmonary function tests, and includes forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC) and diffusing capacity of lung carbon monoxide (DLCO); EF=ejection fraction evaluated via MUGA or ECHO. If subject is unable to perform a PFT, then pulse oximetry should be done instead. These tests will be evaluated within 3 months prior to treatment.

4. CBC includes complete blood count with differential and platelets. Prior to IC9-CAR19 treatment, CBC with differential and platelets must be performed within 24 hours prior to infusion.

5. HIV, HTLV, HBV, HCV testing required for confirmation that no active infection exists; results can be pending at the time of cell procurement; only those samples confirming lack of active infection will be used to generate transduced cells.

6. See section 5.3.1, procurement happens after pre-procurement screening and prior to lymphodepleting chemotherapy.

7. [Complete Metabolic Panel (CMP) + (Mg, P)]: BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P). NOTE: Pre-procurement CMP + (Mg, P) panel should be performed within 7 days of procurement and within 72 hours prior to lymphodepletion. CMP + (Mg, P) panel must be performed within 24 hours prior to iC9-CAR19 cell infusion.

8. To assess status of ALL disease, disease assessment will be performed at baseline (within 30 days prior to enrollment), and prior to lymphodepletion, at 4 and 8 weeks following the infusion, and at later time points (months 3, 6, etc.) as denoted in the Time and Events table above. Lumbar puncture with hematopathology evaluation of CSF is required at screening. At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19

immunophenotyping by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI). If imaging studies are performed at other times after treatment on this study; that data will be collected and information gained will be used for this study. The tests performed at baseline for assessment of ALL should be performed consistently throughout the study.

9. The analyses will be used to monitor function and persistence in peripheral blood and will include functional assays such as in vitro reactivation of PBMCs in subjects for whom the appropriate reagents are available and immunophenotyping; collect before lymphodepletion and prior to cell infusion; also collected at 3-4 hours post cell infusion and during follow up . See section 7.5 for the amount of blood collected/type of tube used for all of the correlative studies.

10. Section 7.5.3 on how to detect a transgene and section 7.4 for additional time points if transgene detected.

11. Replication competent retrovirus (RCR) testing by PCR; if all post treatment samples are negative during the first year, then yearly samples should be archived only. See Section 7.5.2.

12. See Section 7.5.3; collected prior to cell infusion and also collected at 3-4 hours post infusion.

13. Serum from blood drawn for functional studies at baseline and week 4 will be stored for measurement of human anti-mouse antibodies (HAMA). These studies will be performed in the event of a suspected immunologic reaction. See section 7.5.4 for additional details.

14. Serum for cytokine and CRP tests +IL-2R-alpha will be collected before cell infusion, at 3-4 hours after infusion, at weekly intervals through week 4, and then at weeks 6 and 8. After disease evaluation, samples will be collected and appropriately stored at months 3, 6, 9, or 12 for the first year (**Note**: Beyond 8 weeks, the sample for cytokine testing is captured under function and persistence testing time points). After the first year, cytokine evaluation can be performed, if required, on batched samples collected and stored every 6 months for the first 5 yrs and then yearly for up to 15 yrs.

15. Data on all adverse experiences/toxicities regardless of seriousness must be collected for documentation purposes starting <u>at the time of procurement through 4 weeks after dosing of iC9-CAR19 cells</u>. Adverse event data collection will cease in subjects that receive any other hematopoietic cell product or receive therapy for relapse of their primary malignancy. Data on disease status will continue to be collected as appropriate and we will continue to collect data on long-term follow-up for safety of gene transfer (via RCR by PCR).
    a. <u>If CRS signs and symptoms occur</u>, a serum sample (~1 mL) for IL-6 analysis and 10 mL of blood should be collected before instituting treatment for CRS. These samples should be collected regardless of whether the severity of symptoms for

CRS meet criteria for DLT. See section 7.5.5 for additional details. See section 5.1.3 for sample collection procedures to be followed in subjects given AP1903 (0.4 mg/kg) to treat grade 4 CRS or grade 2 or 3 CRS that is unresponsive to standard of care interventions.

b. Serum (~1 mL) and 10 mL of blood should also be collected for serious toxicities that develop that require hospitalization. See Section 7.5.5 for additional details.

16. Certificate of Analysis (CofA) generated at completion of required studies for production/QA of cells

17. Patient reported outcomes (PRO) will be measured by the PROMIS Global Health and Physical Function questionnaires and selected items from the NCI PRO-CTCAE (see section 7.6 and) (optional). PRO questionnaires will only be offered to adult study participants. On the day of infusion, patients will fill out questionnaires prior to infusion. On days 3 and days 5 of week 0 and week 1, subjects will only be offered selected items from the NCI PRO-CTCAE. Symptom questionnaires (selected items from NCI PRO-CTCAE) may also be offered to adult subjects at unscheduled visits or hospital encounters.

18. iC9-CAR19 cells infused within 2-4 days after lymphodepletion.

19. For subjects with grade 4 CRS or grade 2/3 CRS refractory to SOC: AP1903 will be administered i.v. over 2 hrs at 0.4 mg/kg
    - Time points for blood sampling required prior to and after AP1903 administration depicted in section 5.1.3.

    - **Refer to section 7.5.5 for information on sample collection requirements if CRS or severe toxicity occurs**

## 7.2 Screening and Treatment Assessments

### 7.2.1 Pre-procurement

Clinical evaluation: complete history, physical examination including weight and vital signs to assess clinical status, Karnofsky or Lansky performance status (see section 12.1 Appendix A)

Laboratory studies:

- **Pregnancy Test**: serum pregnancy testing will be done in female subjects of childbearing potential (within 72 hours prior to procurement)
- **Pulmonary function tests:** Including FEV1, FVC and DLCO
- **Pulse Oximetry:** Required if PFT cannot be performed by subject
- **Ejection Fraction**
- **HIV, HTLV, HBV, HCV** testing required (see footnote #5 of Time and Events table)
- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, magnesium), (P, phosphorus)]

Disease Assessment (ALL): At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of bone marrow or blood by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. **Lumbar puncture required at this screening**. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI). **The tests performed at baseline for assessment of ALL should be performed consistently throughout the study.**

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE.

**Procurement**: See Section 5.2.1; to take place approximately 2-5 weeks prior to lymphodepleting chemotherapy. Subjects should be followed for safety from this point forward.

### 7.2.2 Pre-Lymphodepletion

Clinical evaluation: History, physical examination including height (baseline only), weight and vital signs, Karnofsky or Lansky performance status (see section 12.1 Appendix A), Mini-mental status exam [MMSE; adults only; see section 12.5 Appendix E)]

Laboratory studies (within 72 hours of lymphodepletion):

- **Pregnancy Test**: serum pregnancy testing will be done in female subjects of childbearing potential
- **Pulse Oximetry**
- **CBC with differential and platelets:**

- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **IgG & IgA**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies:** Including in vitro reactivation of PBMCs in subjects for whom the appropriate reagents are available and immunophenotyping
  - **Replication competent retrovirus (RCR) testing:** by PCR
  - **Quantitative PCR to test for integrated transgene**
  - **HAMA testing**

Disease Assessment (ALL): At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or bone marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study.

Toxicity: Toxicity will be assessed according to the NCI CTCAE v. 4.03. Refer to section 8.2 for details

CofA: Generated at completion of required studies for production/QA of cell (available prior to planned infusion)

### 7.2.3 Lymphodepletion

Subjects will receive fludarabine 25 mg/m$^2$/day i.v. over 30 minutes administered for 3 consecutive days followed by cyclophosphamide 900 mg/m$^2$ i.v. over 1 hr on the 4$^{th}$ day.

See sections 5.2.2 and 6.3 for details.

### 7.2.4 Cellular Treatment (D1 of Week 0)

iC9-CAR19 cells infused 2-4 days after lymphodepletion ( iC9-CAR19+ T cells/kg as described in sections 5.2.3 and 5.2.4)

Clinical evaluation: History, physical examination including weight and vital signs to assess clinical status, Karnofsky or Lansky performance status (see section 12.1 Appendix A), MMSE (adults only; see section 12.5 Appendix E)

Laboratory studies (assess within 24 hours prior to cell infusion):

- **Pulse Oximetry**
- **CBC with differential and platelets:**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **Blood Sample (pre/post) for Correlative Studies (see section 7.5)**
  - **Function and persistence studies:** 3-4 hours post infusion

- **Quantitative PCR to test for integrated transgene:** 3-4 hours post infusion
- **Cytokines + CRP + IL-2R alpha** (collect pre and post infusion samples) The post infusion sample should be collected within 3 or 4 hours after the infusion

Toxicity of cell infusion: Toxicity will be assessed according to the NCI CTCAE v.4.03.
Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE. This will be collected on the day of infusion prior to infusion.

## 7.3 Short-term Follow-up

### 7.3.1 D3 and D5 of Wk 0 and Wk 1

Clinical evaluation: History, physical examination including weight and vital signs to assess clinical status (see section 12.1 Appendix A), MMSE (adults only; see section 12.5 Appendix E)
Laboratory studies:

- **Pulse Oximetry**
- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]

Toxicity of cell infusion: Toxicity will be assessed according to the NCI CTCAE v.4.03.
Patient reported outcome questionnaires for adult subjects (optional): Selected questions from NCI PRO-CTCAE

### 7.3.2 D1 Week 1

Clinical evaluation: History, physical examination including weight and vital signs to assess clinical status, MMSE (adults only; see section 12.5 Appendix E)
Laboratory studies:

- **Pulse Oximetry**
- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **Quantitative PCR to test for integrated transgene**
  - **Cytokine testing + CRP + IL-2R alpha**

Toxicity of cell infusion: Toxicity will be assessed according to the NCI CTCAE v.4.03.
Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.3 D1 Week 2

Clinical evaluation: history, physical examination including weight and vital signs to assess clinical status, Karnofsky or Lansky performance status (see section 12.1 Appendix A), MMSE (adults only; see section 12.5 Appendix E).
Laboratory studies:

- **Pulse Oximetry**
- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **Quantitative PCR to test for integrated transgene**
  - **Cytokine testing + CRP + IL-2R alpha**

Toxicity of cell infusion: Toxicity will be assessed according to the NCI CTCAE v.4.03.
Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.4 D1 Week 3

Clinical evaluation: History, physical examination including weight and vital signs to assess clinical status, MMSE (adults only; see section 12.5 Appendix E)

- **Pulse Oximetry**
- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies:**
  - **Quantitative PCR to test for integrated transgene:**
  - **Cytokines +CRP + IL-2R alpha**

Toxicity of cell infusion: Toxicity will be assessed according to the NCI CTCAE v.4.03.
Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.5 D1 Week 4

Clinical evaluation: History, physical examination including weight and vital signs to assess clinical status, Karnofsky or Lansky performance status (see section 12.1 Appendix A), MMSE (adults only; see section 12.5 Appendix E)
Laboratory studies:

- **Pulse oximetry**
- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **IgG**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **HAMA testing**
  - **Quantitative PCR to test for integrated transgene**
  - **Cytokine testing + CRP + IL-2R alpha**

Disease Assessment (ALL): At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow

cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study.

Toxicity of cell infusion: Toxicity will be assessed according to the NCI CTCAE v.4.03.

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.6 D1 Week 6

Clinical evaluation: History, physical examination including weight and vital signs to assess clinical status (NCI CTCAE v4.03), Karnofsky or Lansky performance status (see section 12.1 Appendix A),

Laboratory studies:

- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **Quantitative PCR to test for integrated transgene**
  - **Cytokine testing + CRP + IL-2R alpha**

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.7 D1 Week 8

Clinical evaluation: History, physical examination including weight and vital signs to assess clinical status (NCI CTCAE v4.03), Karnofsky or Lansky performance status (see section 12.1 Appendix A)

Laboratory studies:

- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **IgG**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **Quantitative PCR to test for integrated transgene**
  - **Cytokine testing + CRP + IL-2R alpha**

Disease Assessment (ALL): At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or bone marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates.

Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study.

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.8 D1 Month 3

Clinical evaluation: history, physical examination to include weight and vital signs to assess clinical status (NCI-CTCAE v4.03), Karnofsky or Lansky performance status (see section 12.1 Appendix A).

Laboratory studies:

- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **IgG**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **RCR testing:** by PCR
  - **Quantitative PCR to test for integrated transgene**

Disease Assessment (ALL): At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study.

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.9 D1 Month 6

Clinical evaluation: history, physical examination to include weight and vital signs to assess clinical status (NCI-CTCAE v4.03), Karnofsky or Lansky performance status (see section 12.1 Appendix A).

Laboratory studies:

- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **IgG**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **RCR testing:** by PCR
  - **Quantitative PCR to test for integrated transgene**

Disease Assessment (ALL): At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study.
Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.10 D1 Month 9

Clinical evaluation: history, physical examination to include weight and vital signs to assess clinical status (NCI-CTCAE v4.03), Karnofsky or Lansky performance status (see section 12.1 Appendix A).
Laboratory studies:

- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **IgG**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **Quantitative PCR to test for integrated transgene**

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.11 D1 Month 12

Clinical evaluation: history, physical examination to assess clinical status (NCI-CTCAE v4.03), Karnofsky or Lansky performance status (see section 12.1 Appendix A).
Laboratory studies:

- **CBC with differential and platelets**
- **[CMP + (Mg, P)]:** [BUN, creatinine, Na, K, Cl, $CO_2$, glucose, Ca, albumin, total protein, total bilirubin, ALT, AST, and alkaline phosphatase + (Mg, P)]
- **IgG**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies**
  - **RCR testing:** by PCR
  - **Quantitative PCR to test for integrated transgene**

Disease Assessment (ALL): At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for

Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study.

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

### 7.3.12 Every 6 months X 4 years

Clinical evaluation: history, annual physical examination (each year until 5 years after last cell infusion) to include height (pediatric subjects only), weight and vital signs to assess clinical status (NCI-CTCAE v4.03), Karnofsky or Lansky performance status (see section 12.1 Appendix A).

Laboratory studies:

- **CBC with differential and platelets**
- **IgG**
- **Blood Sample for Correlative Studies (see section 7.5)**
  - **Function and persistence studies (see section 7.5.1)**
  - **RCR testing:** by PCR **(see section 7.5.2**)
  - **Quantitative PCR to test for integrated transgene (see section 7.5.3)**
  - **Cytokine testing + CRP + IL-2R alpha (see footnote #14 in table of assessments)**

Disease Assessment in follow up (ALL): **Perform only when suspicion of relapse per investigator discretion as clinically indicated.** At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study follow up period.

Patient reported outcome questionnaires for adult subjects (optional): PROMIS Global Health Scale, PROMIS Physical Function, and selected questions from NCI PRO-CTCAE

## 7.4 Long Term Follow-Up

If a transgene is detected, assays (quantitative PCR (see Section 7.5.3) and function and persistence studies) will continue every 6 months up to year 5. Otherwise subjects will be followed yearly for a total of 15 years, as mandated by FDA. Additional visits will be obtained as clinically indicated. If there is suspicion of relapse during long-term follow-up, disease assessments will occur according to other time points of the study. At a minimum, disease assessment should include bone marrow aspirate and biopsy as well as peripheral blood assessment for percentage of circulating lymphoblasts along with CD19 immunophenotyping of blood or marrow by either flow cytometry or IHC. Minimal residual disease testing by multicolor flow cytometry (for all subjects) with RT-PCR for

quantitation of BCR-ABL transcripts (for Ph+ ALL subjects only) will be performed on the first pull of all bone marrow aspirates. Imaging or sampling of known or suspected extramedullary leukemia is at the discretion of the treating physician. The choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI) and should be performed consistently during the study follow up period. Function and persistence testing, RCR by PCR, and quantitative PCR along with a brief history, physical exam, IgG measurement and performance status to assess clinical status (NCI-CTCAE v4.03) should be performed.

## 7.5 Correlative Studies: Tests of Function, Persistence and Safety

The following investigations will be used to monitor function and persistence in peripheral blood and safety of transduced T- cells at time-points indicated in the Time and Events Table. Unless otherwise specified, 46 mLs in Sodium Heparin Green top tubes and 3mLs in a red top tube of blood will be collected at each time-point. A maximum of 50 mL of blood for adults or 2 mL/kg body weight of blood for children will be drawn on any one day for these assays. If a subject's hemoglobin is less than 8.0 g/dL at any of the evaluation times, the amount of blood drawn for the evaluation will be reduced and may be obtained over more than one venipuncture, if necessary. If there is insufficient blood, RCR testing and Quantitative real-time PCR will be the first priorities (RCR testing > T cell frequency/persistence > ELISPOT/multimer assays for cytokines > cytotoxicity assays for anti-tumor specificity and cytotoxic T lymphocyte precursor assays). **Note**: The studies are not necessarily performed at that time of blood collection. Studies will be conducted depending on the availability of the subject and the ability to safely draw the amount of blood needed for the studies. The time points given are approximate as subjects may not always be able to keep appointments. Every effort will be made, however, to obtain studies on schedule. Residual samples will be stored for any future study-related assays. **Cytokines**: Our standard array for cytokine determination includes 27-cytokines (UNC immune monitoring Core) which among others include: IL-2, IFNγ, TNFα, IL-6, IL-10, IL-13, IL-1Rα, IL-8, IL-12, IL-15, and GM-CSF.

### 7.5.1 Function and Persistence Studies

These will include functional assays such as *in vitro* reactivation of PBMCs in subjects for whom the appropriate reagents are available. Immunophenotyping will also be conducted when applicable.

### 7.5.2 RCR Testing by PCR

RCR testing will be done on samples collected pre-infusion, 3 months, 6 months and 12 months after treatment. If all post treatment assays are negative during the first year, then further yearly samples should be archived. If any post treatment samples are positive, further analysis of the RCR and more extensive subject follow-up should be undertaken in consultation with the FDA.

Aliquot of cells and serums will also be archived for use in future studies for RCR as required by the FDA or NIH's RAC.

### 7.5.3 Quantitative PCR to Test for Integrated Transgene

Quantitative real-time PCR to detect retroviral integrants will be collected pre-infusion, 3-4 hours post infusion, 1, 2, 3, 4, 6 and 8 weeks post- T cell infusion and then at 3, 6, 9, and 12 months then yearly for a total of 15 years. PCR to detect retroviral integrant clonality and integrant locus will also be done at the first occurrence (if the transgene is detected at >0.5%) and then every 3 months for the first year. If the transgene is still detected at > 0.5% level assays will continue every 6 months up to year 5. If the level of detection is less than 0.5% samples will be collected annually and may be archived. Follow up will continue for 15 years.

### 7.5.4 HAMA Testing

Serum from blood drawn for functional studies at baseline and at week 6 will be stored for measurement of HAMA. These studies will be performed in the event of a suspected immunologic reaction.

### 7.5.5 Sample Collection Requirements if CRS or Severe Toxicity Occurs

A serum sample (~1 mL) for IL-6 analysis and ~10 mL of blood should be collected in any subject who develops signs and symptoms of CRS regardless of whether their symptoms meet severity requirements for DLT. This sample should be collected **before** treatments are administered to alleviate CRS symptoms. Subsequent samples may be collected at the discretion of the investigator.

In addition, a serum sample (~1 mL) for IL-6 analysis and ~10 mL of blood should be collected in any subject who develops a SAE (ie, requiring hospitalization) related to the cell infusion. Subsequent samples may be collected at the discretion of the investigator.

### 7.5.6 Other Tissue Studies

If biopsy of accessible lymph nodes is required at any time during the first year after treatment, a sample of this will be used to assess presence of transduced peripheral blood T-cells in association with the tumor.

When bone marrow trephine biopsy or biopsy of suspected extramedullary leukemia (including CSF evaluation of suspected CNS relapse) is required at any time during the first year after treatment, a sample of this will be used to assess presence of transduced peripheral blood T- cells in association with the tumor.

## 7.6 Patient Reported Outcome Measures

Patient reported outcome measures will be collected on adult study participants who agree to fill out questionnaires at the scheduled clinic visits. The PROMIS questionnaires corresponding to Global Health (PROMIS GHS SF v1.0-1.1) and Physical Function (PROMIS Physical Function SF20a) (www.nihpromis.org) and selected symptom questions from the NCI PRO-CTCAE will be administered (See Appendix D: Patient Reported Outcome Surveys). In addition, the symptom questionnaire (selected symptom

questions from the NCI PRO-CTCAE) will also be offered to adult subjects during any extra unscheduled study encounters such as unscheduled clinic visits or hospitalizations.

Adult subjects who experience disease progression following an initial infusion will be asked to complete a final set of questionnaires if they are willing.

## 7.7 Biobank Repository

Subjects participating in this trial must consent to allow researchers to store their biological specimens. Participants in this trial will also be required to sign a separate HIPAA authorization form to allow investigators to review their medical records.

IC9-CAR19 T-cells will be stored at the GMP facility according to standard procedures. Other specimens collected during this study as described in section 7.5 of this protocol will be stored at the University of North Carolina at Chapel Hill in the immunotherapy laboratory in locked liquid nitrogen tanks with controlled access. The samples will be labeled with the study ID number and date and time of sample collection.

The purpose of this repository or biobank is to store samples for immediate or future analyses to answer study-related questions, not to increase general knowledge outside the parameters of the study. These goals will be accomplished using several different kinds of specimens collected during the study as described. These specimens include:

- Tissue samples from standard of care biopsies of the bone marrow and/or extramedullary tumor
- Unused iC9-CAR19 T-cells in a subject who has already undergone a cell infusion
- Unused additional material collected during procurement (peripheral blood mononuclear cells)
- Leftover tissue obtained for any standard of care procedure that may provide additional information about iC9-CAR19 T-cell therapy

For example, analyses will be performed to assist in determining if the iC9-CAR19 T-cells are associated with the tumor and if the iC9-CAR19 T-cells are present.

Tissues donated from bone marrow biopsies, extramedullary tumor biopsies, or any leftover tissue obtained through standard of care procedures that may provide additional information about iC9-CAR19 T-cell therapy will be stored for 15 years in the repository. After 15 years, these samples will be destroyed. IC9-CAR19 T-cells will be kept for 5 years and destroyed thereafter.

Information about the patient's disease will be linked to the specimens stored in the repository database. Immunotherapy laboratory-associated research staff, LCCC Bioinformatics staff who support the database and the LCCC Data Warehouse, and researchers with IRB-approval for access to personal health information for each subject in this study will be able to link specimens to relevant medical information. Some results from laboratory analyses that occurred during the patient's participation in the clinical

study may also be included. This information may be important for understanding how the patient's cancer developed and responded to treatment.

**Storage Time:**

- iC9-CAR19 T-cells will be stored for 5 years. After 5 years they will be destroyed
- Tissue obtained from bone marrow biopsies, extramedullary tumor biopsies, or any leftover tissue obtained through standard of care procedure that may provide additional information about iC9-CAR19 T-cell therapy will be stored for up to 15 years

## 7.8 Assessment of Safety

All subjects who undergo cell procurement will be included in the safety analysis. Each subject will be assessed periodically for the development of any toxicity according to the Time and Events table (section 7.1). Toxicity will be assessed according to the NCI CTCAE v4.

## 7.9 Assessment of Efficacy

To assess disease status, bone marrow aspirates and biopsies, with aspirates evaluated for MRD, will be performed at baseline and at 4 and 8 weeks following the infusion. If extramedullary disease has been previously imaged, the choice of imaging will depend on what studies have been most informative in following the subject's disease (i.e., PET, CT scans, MRI, nuclear imaging). If imaging studies are performed at other times after treatment on this study; that data will be collected and information gained will be used for this study.

### 7.9.1 Measurement of Disease

This study will use the National Cancer Center Network (NCCN ALL Response Criteria Version 1.2014 available at http://www.tri-kobe.org/nccn/guideline/hematologic/english/all.pdf)

| **Response Criteria for Blood and Bone Marrow** |
|---|
| Complete Response (CR)<br>• No circulating blasts or extramedullary disease<br>→No lymphadenopathy, splenomegaly, skin/gum infiltration/testicular mass/CNS involvement<br>• Trilineage hematopoiesis and < 5% blasts<br>• ANC > 1,000/µl<br>• Platelets >100,000/µl<br>• No recurrence for 4 weeks<br>Complete Response with incomplete recovery of counts (CRi)<br>• Recovery of platelets but < 100,000 or ANC < 1000/µl<br>ORR (ORR=CR + CRi)<br>Refractory disease<br>• Failure to achieve CR at the end of week 4- or 8-week assessments<br>Progressive disease |

| |
|---|
| • Increase of at least 25% in the absolute number of circulating or bone marrow blasts or development of extramedullary disease<br>Relapsed disease<br>Reappearance of blasts in the blood or bone marrow (>5%) or in any extramedullary site after a CR |
| **Response Criteria for CNS Disease** |
| • CNS remission: Achievement of CNS-1 status in a subject with CNS-2 or CNS-3 status at diagnosis<br>• CNS relapse: New development of CNS-3 status or clinical signs of CNS leukemia such as facial nerve palsy, brain/eye involvement, or hypothalamic syndrome |
| Classification of CNS status |
| • CNS-1: No lymphoblasts in cerebrospinal fluid (CSF) regardless of WBC<br>• CNS-2: WBC < 5/µl in CSF with presence of lymphoblasts<br>• CNS-3: WBC ≥ 5/µl in CSF with presence of lymphoblasts |
| **Response Criteria for Mediastinal Disease** |
| Complete Response (CR)<br>• Complete resolution of mediastinal enlargement by CT<br>Complete Response Unconfirmed (Cru)<br>• Residual mediastinal enlargement that has regressed by > 75% in sum of the products of the greatest perpendicular diameters (SPD)<br>Partial Response (PR)<br>• >50% decrease in the SPD of the mediastinal enlargement<br>Progressive Disease (PD):<br>• > 25% increase in the SPD of the mediastinal enlargement<br>No Response (NR)<br>• Failure to qualify for PR or PD<br>Relapse<br>• Recurrence of mediastinal enlargement after achieving CR or CRu |

Minimum residual disease (MRD): Leukemic cells below the threshold of detection by conventional morphologic methods. Subjects who achieve a CR by morphologic assessment can potentially harbor a large number of leukemic cells in the bone marrow. A strong correlation exists between MRD and risks of relapse.

## 7.10 Criteria for Removal from Protocol Therapy

### 7.10.1 Criteria for Removal from Protocol Therapy, but Continued Long-term Follow-up

- Progressive disease
- DLT noted during disease re-evaluation
- Any subject who develops Grade 4 non hematologic toxicity after treatment is removed from the trial.
- Grade 3-4 allergic reaction to AP1903
- Positive pregnancy test
- Parent of subject or subject decides to withdraw from therapy
- Completion of planned therapy and follow up samples
- Physician decision

### 7.10.2 Off Study Criteria

- Death
- Lost to follow up
- Withdrawal of consent for any further data submission

# 8.0 ADVERSE EVENTS

## 8.1 Definitions

### 8.1.1 Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence (e.g., an abnormal laboratory finding, symptom, or disease temporally associated with the use of a drug or cellular therapy) in a subject or clinical investigation subject administered a pharmaceutical/cellular product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not related to the medicinal product.

Hospitalization for elective surgery or routine clinical procedures that are not the result of an AE (e.g., surgical insertion of central line) need not be considered AEs and should not be recorded as an AE. Disease progression should not be recorded as an AE, unless it is attributable by the investigator to the study therapy.

### 8.1.2 Suspected Adverse Reaction (SAR)

A suspected adverse reaction (SAR) is any AE for which there is a *reasonable possibility* that the drug or cellular product is the cause. *Reasonable possibility* means that there is evidence to suggest a causal relationship between the drug and the AE. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug or cellular product.

Causality assessment to a study drug or cell infusion is a medical judgment made in consideration of the following factors: temporal relationship of the AE to study drug or cellular product exposure, known mechanism of action or side effect profile of study treatment, other recent or concomitant drug exposures, normal clinical course of the disease under investigation, and any other underlying or concurrent medical conditions. Other factors to consider in considering drug as the cause of the AE:

- Single occurrence of an uncommon event known to be strongly associated with drug or cellular product exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome)
- One or more occurrences of an event not commonly associated with drug or cellular product exposure, but otherwise uncommon in the population (e.g., tendon rupture); often more than once occurrence from one or multiple studies would be needed before the sponsor could determine that there is *reasonable possibility* that the drug or cell infusion caused the event.

- An aggregate analysis of specific events observed in a clinical trial that indicates the events occur more frequently in the drug or cellular product treatment group than in a concurrent or historical control group

### 8.1.3 Unexpected AE or SAR

An AE or SAR is considered unexpected if the specificity or severity of it is not consistent with the applicable product information (e.g., as provided in the IND and/or Investigator's Brochure (IB) for an unapproved investigational product or package insert/summary of product characteristics for an approved product). Unexpected also refers to AEs or SARs that are mentioned in the IB or IND as occurring with a class of drugs or cellular product or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

### 8.1.4 Serious AE or SAR

An AE or SAR is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death;
- Is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- Requires inpatient hospitalization (>24 hours) or prolongation of existing hospitalization;*
- Results in congenital anomaly/birth defect;
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions;
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. For reporting purposes, also consider the occurrences of pregnancy as an event which must be reported as an important medical event.
- Grade 4 or 5 cell product infusion reactions, CRS or neurologic toxicity

*Hospitalization for anticipated or protocol specified procedures such as administration of chemotherapy, central line insertion, metastasis interventional therapy, resection of primary tumor, or elective surgery, will not be considered serious adverse events.

Pregnancy that occurs during the study must also be reported as an SAE.

## 8.2 Documentation of non-serious AEs or SARs

For non-serious AEs or SARs, documentation must begin at the time of procurement when the consent form is signed by the subject prior to procurement and continues through the 4week follow-up period after cellular treatment is discontinued. Any AEs or SARs experienced by the subject related to these procedures (procurement, lymphodepletion, and cell infusion) must also be documented. The DLT assessment

period will start at the time of the cell infusion through the 4-week follow up period after the infusion.

Collected information should be recorded in the Case Report Forms (CRF) for that subject. Please include a description of the event, its severity or toxicity grade, onset and resolved dates (if applicable), and the relationship to the study drug. Documentation should occur at least monthly.

## 8.3 SAEs or Serious SARs

### 8.3.1 Timing

After informed consent but prior to initiation of study medications, only SAEs caused by a protocol-mandated intervention will be collected (e.g. SAEs related to invasive procedures such as biopsies, medication washout).

For any other experience or condition that meets the definition of an SAE or a serious SAR, recording of the event must begin at procurementand continue through the 30 day follow-up period after treatment is discontinued.

### 8.3.2 Documentation and Notification

SAEs or Serious SARs must be recorded in the SAE console within Oncore™ for that subject within 24 hours of learning of its occurrence. The Regulatory Associate and Medical Monitor must be notified via email of all SAEs within 24 hours of learning of its occurrence.

### 8.3.3 Reporting

**IRB Reporting Requirements:**

UNC**:**

- The UNC-IRB will be notified of all SAEs that qualify as an Unanticipated Problem as per the UNC IRB Policies using the IRB's web-based reporting system (see section 9.5.3) within 7 days of the Investigator becoming aware of the problem. Please note, these events must be reported to the sponsor within 24 hours of learning of the occurrence.

Pregnancy

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is on study, or within 28 days of the subject's last dose of study should be recorded as SAEs. The subject is to be discontinued immediately from protocol therapy. The female subject should be referred to an obstetrician-gynecologist, preferably one experienced in reproductive toxicity for further evaluation and counseling.

The Investigator will follow the female subject until completion of the pregnancy, and must document the outcome of the pregnancy (either normal or abnormal outcome) and report the condition of the fetus or newborn to the Study Coordinator. If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator

should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE.

**FDA Expedited Reporting requirements for studies conducted under an IND:**
A sponsor must report any suspected adverse reaction that is both serious and unexpected and related to the cellular product to the FDA. The sponsor must report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the cellular product and the adverse event, such as:

- A single occurrence of an event that is uncommon and known to be strongly associated with cell infusion exposure (e.g., angioedema, hepatic injury, Stevens-Johnson Syndrome);
- One or more occurrences of an event that is not commonly associated with cellular product exposure, but is otherwise uncommon in the population exposed to the drug (e.g. tendon rupture);
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the cellular product treatment group than in a concurrent or historical control group.

The sponsor must submit each IND safety report on NIH OSP/GeMCRIS. Each notification to FDA must bear prominent identification of its contents, i.e., "IND Safety Report," and must be transmitted to the review division that has the responsibility for review of the IND. For this study, the review division is the Center for Biologics Evaluation and Research. In each IND safety report, the sponsor must identify all IND safety reports previously submitted to FDA concerning a similar suspected adverse reaction, and must analyze the significance of the suspected adverse reaction in light of previous, similar reports or any other relevant information.

*Timing*
FDA must be notified of potential serious risks within 15 calendar days after the sponsor determines the event requires reporting. FDA must be notified of unexpected fatal or life-threatening suspected adverse reactions as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. The sponsor must be notified of the SAE by the investigator within 24 hours of the event. If the results of a sponsor's investigation show that an adverse event not initially determined to be reportable is reportable, the sponsor must report such suspected adverse reaction in an IND safety report as soon as possible, but in no case later than 15 calendar days after the determination is made.

*Follow-up*
The sponsor must promptly investigate all safety information it receives. Relevant follow-up information to an IND safety report must be submitted as soon as the information is available and must be identified as such, i.e., "Follow-up IND Safety Report." Additionally, upon request from FDA, the sponsor must submit to FDA any additional data

or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

*Notification of Investigators*
The sponsor must notify all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

*Process*
If the sponsor deems that an event is both a serious adverse reaction (SAR) AND unexpected, it must also (in addition to Oncore) be recorded on the MedWatch Form 3500A as per 21 CFR 312.32. Unexpected adverse events or adverse reaction refers to an event or reaction that is not listed in the investigator's brochure/IND or is not listed at the specificity or severity that has been observed; or if an investigator's brochure is not required or available, is not consistent with the risk information described in the general investigation plan or elsewhere in the current IND application.

The MedWatch form should be submitted on MedWatch by the study coordinator.

The MedWatch 3500a form can be accessed at:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm.
(Please be sure and access form 3500a, and not form 3500).

*Additional Reporting Requirements*
The following additional items must be reported via IND safety report:

- *Findings from other studies.* The sponsor must report any findings from epidemiological studies, pooled analysis of multiple studies, or clinical studies, whether or not conducted under an IND, and whether or not conducted by the sponsor, that suggest a significant risk to humans exposed to the drug.
- *Findings from animal or in vitro testing.* The sponsor must report any findings from animal or *in vitro* testing, whether or not conducted by the sponsor, that suggest a significant risk in humans exposed to the drug, such as reports of mutagenicity, teratogenicity, or carcinogenicity, or reports of significant organ toxicity t or near the expected human exposure.
- *Increased rate of occurrence of serious suspected adverse reactions.*

*Additional Guidance*
Please refer to 21CFR312.32 and "Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies" for additional information and reporting requirements. All IND Safety Reports will be submitted in accordance with these regulations/guidances.

**NIH Recombinant DNA Advisory Committee (RAC)**

Principal Investigators must submit, in accordance with Appendix M-I-C-4-a and Appendix M-I-C-4-b a written report on:

- Any serious adverse event that is both unexpected and associated with the use of the gene transfer product (i.e., there is reasonable possibility that the event may have been caused by the use of the product; investigators should not await definitive proof of association before reporting such events
- Any finding from tests in laboratory animals that suggests a significant risk for human research participants including reports of mutagenicity, teratogenicity, or carcinogenicity.

This report must be clearly labeled as a "Safety Report" and must be submitted to the NIH Office of Science Policy (NIH OSP).

*Timing*

Any serious adverse event that is fatal or life-threatening that is unexpected, and associated with the use of the gene transfer product must be reported to the NIH OSP as soon as possible, but not later than 7 calendar days after the sponsor's initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the gene transfer product, but are not fatal or life-threatening, must be reported to the NIH OSP as soon as possible, but not later than 15 calendar days after the sponsor's initial receipt of the information. If, after further evaluation, an adverse event initially considered not to be associated with the use of the gene transfer product is subsequently determined to be associated, then the event must be reported to the NIH OSP within 15 days of the determination.

*Follow-up*

Relevant additional clinical and laboratory data may become available following the initial serious adverse event report. Any follow-up information relevant to a serious adverse event must be reported within 15 calendar days of the sponsor's receipt of the information. If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the gene transfer product, that event shall be reported to the NIH OSP within 15 calendar days of the determination.

*Process*

The UNC study coordinator will be responsible for notifying the Regulatory Associate and IND Specialist within 48 hours should any reportable event occur. The IND Specialist will submit the report to the NIH RAC within the required time period. The three alternative mechanisms for reporting serious adverse events to the NIH OSP are:

- By email to: HGTprotocols@mail.nih.gov
- Fax to (301) 496-9839
- By mail to the Office of Science Policy, National Institutes of Health, MSC 7985, 6705 Rockledge Drive, Suite 750, Bethesda, Maryland 20892-7985

**Institutional Biosafety Committee (IBC)**

In addition to the local IRB, any qualifying serious adverse events (SAEs) must be reported to the Institutional Biosafety Committee (IBC). The IBC is responsible for reviewing

recombinant DNA research conducted at or sponsored by the institution for compliance with NIH Guidelines as specified in Section III, Experiments covered by the NIH Guidelines and approving those research projects that are found to conform with the NIH Guidelines. As such, the IBC is charged with ensuring compliance with all surveillance, data reporting and adverse event reporting requirements set forth in the NIH Guidelines. The UNC study coordinator will be responsible for notifying the Regulatory Associate within 48 hours should any reportable event occur.

## 8.4 Data and Safety Monitoring Plan

The Principal Investigators will provide continuous monitoring of subject safety in this trial with periodic reporting to the Data and Safety Monitoring Committee (DSMC). The Principal Investigators responsible for the care of adult and pediatric subjects in this study will also oversee the conduct of safety data review meetings on a regular basis. Meetings/teleconferences will be held at a frequency dependent on study accrual, and in consultation with the study Biostatistician. These meetings will include the investigators as well as study coordinators, clinical research associates, regulatory associates, data managers, biostatisticians, and any other relevant personnel the principal investigators may deem appropriate. At these meetings, the research team will discuss all issues relevant to study progress, including enrollment, safety, regulatory issues, data collection, etc. An agenda and minutes will be generated for each meeting to document attendees and subject data reviewed. Bi-weekly safety review meetings will be instituted as soon as a subject(s) enters the study, has received iC9-CAR19 cell therapy on D0 and is undergoing clinical assessments in the 4-week evaluation period following the cell infusion. For the dose finding study, a safety data review meeting will be convened approximately 1-2 weeks after each subject has completed iC-CAR19dosing and scheduled to coincide with the study's bi-weekly safety meetings. In addition, recurring monthly meetings will be held to review all available data collected in the study up to that point. Ad hoc meetings will be convened if an unexpected safety concern is identified during the conduct of the trial. An agenda and minutes will be generated for each meeting to document attendees and subject data reviewed. An adult and pediatric clinical investigator, a study coordinator, a GMP facility representative, and a data manager are required attendees for all safety meetings held to review safety data collected in the dose finding Phase I study. Documentation of these meetings will be kept in the study file.

<u>Dose Escalation Meetings</u>

Subjects will be monitored on a weekly basis until they have completed the DLT assessment evaluations. When there is sufficient information available for making an informed dose escalation/de-escalation decision (i.e., at least 3 subjects at the same dose level have completed the DLT assessment period evaluations per 3+3 rules) a dose escalation/de-escalation decision meeting will be held in conjunction with the bi-weekly safety meeting. Decisions/outcomes of each safety review and dose escalation meeting will be documented and placed in the study file. An adult and pediatric clinical investigator, a study coordinator, and the statistician must attend the dose escalation/de-escalation decision meetings.

The UNC LCCC Data and Safety Monitoring Committee (DSMC) will review the study on a regular (quarterly to annual) basis, with the frequency of review based on risk and complexity as determined by the UNC Protocol Review Committee. As such, phase I trials must undergo quarterly reviews by the full board. AEs contributing to DLTs should be reported on a monthly basis The Principal Investigator will be responsible for submitting the following information for review: 1) safety and accrual data including the number of subjects treated; 2) significant developments reported in the literature that may affect the safety of participants or the ethics of the study; 3) preliminary response data; and 4) summaries of team meetings that have occurred since the last report. Findings of the DSMC review will be disseminated by memo to the UNC PI, PRC, and the UNC IRB and DSMB.

# 9.0 STATISTICAL CONSIDERATIONS

## 9.1 Study Design/Study Endpoints

This single center, open-label phase I dose escalation trial seeks to determine if CAR-T cells targeting the CD19 antigen and containing the inducible caspase 9 safety switch can be safely administered to adult and pediatric subjects with relapsed or refractory CD19+ ALL. At least adult 6 subjects will be assigned in the adult dose-escalation portion of the study and followed for at least 4 weeks before the study in pediatric subjects can begin. The 3+3 design will be used in adult subjects and an independent study using the 3+3 design will be run in pediatric subjects. The starting dose of $5 \times 10^5$ transduced cells/kg (dose level 1) will enroll 3 adult subjects in the initial cohort. If there are no DLTs within 4 weeks of the cell infusion in these 3 subjects, then the next cohort will evaluate $1 \times 10^6$ transduced cells/kg in adults. If there is toxicity in 1/3 patients in the initial cohort, the cohort will be expanded to enroll up to 6 adult patients. If dose level 1 is determined to be above the TCD, de-escalation would occur to dose level -1 where subjects would receive $1 \times 10^5$ transduced cells/kg.

The 3+3 design is described as follows

| Number of Subjects with DLT | Action |
|---|---|
| 0 out of 3 subjects | Escalate to next dose level |
| 1 out of 3 subjects | Accrue 3 additional evaluable subjects at current dose level |
| 1 out of 6 subjects | Escalate to next dose level |
| 2 or more subjects in a dosing cohort | TCD has been exceeded. Expand a prior cohort tested at a lower dose level unless 6 subjects have been assigned to that dose already |

The tolerable cell dose (TCD) is defined as the dose at which 0.20 of patients experience DLT. The estimated TCD is the dose where 0 – 1 out of 6 patients experienced DLT.

### 9.2 Sample Size and Accrual

The number of adult and pediatric subjects required for this study will depend on DLTs we observe. Our expectation is that 9 adult and 9 pediatric subject will be needed, however as many as 18 adult and 18 pediatric subject might be required.

### 9.3 Data analysis

Descriptive statistics will be utilized to summarize toxicity overall and by dose.

## 10.0 STUDY MANAGEMENT

### 10.1 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the subject will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the subject and the investigator is assured that the subject understands the implications of participating in the study, the subject will be asked to give consent to participate in the study by signing an IRB-approved consent form.

Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject and by the person who conducted the informed consent discussion.

### 10.2 Required Documentation

Before the study can be initiated at any site, the following documentation must be provided to the Clinical Protocol Office (CPO) at the University of North Carolina.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any sub-investigators who will be involved in the study.
- Form FDA 1572 CAP and CLIA Laboratory certification numbers and institution lab normal values
- Executed clinical research contract

### 10.3 Registration Procedures

All patients must be registered by the Cellular Immunotherapy study coordinator at UNC before enrollment to study. Prior to registration, eligibility criteria must be confirmed with the Cellular Immunotherapy Study Coordinator.

### 10.4 Data Management and Monitoring/Auditing

The UNC LCCC will serve as the coordinating center for this trial. Data will be collected through a web based clinical research platform, OnCore®.

All data will be collected and entered into OnCore® by Clinical Research Associates (CRAs) from UNC LCCC.

The sponsor will provide direct access to source data/documents for trial-related monitoring, audits, IRB/IEC review, and regulatory inspection. As an investigator initiated study, this trial will also be audited by the LCCC compliance committee every six or twelve months.

### 10.5 Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study subject requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

#### 10.5.1 Emergency Modifications

UNC and Affiliate investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior UNC IRB/IEC approval/favorable opinion.

For any such emergency modification implemented, a UNC IRB modification form must be completed by UNC Research Personnel within five (5) business days of making the change.

#### 10.5.2 Single Subject/Subject Exceptions

For Institutions Relying on UNC's IRB:
Any request to enroll a single subject who does not meet all the eligibility criteria of this study requires the approval of the UNC Principal Investigator and the UNC IRB.

#### 10.5.3 Other Protocol Deviations/Violations

According to UNC's IRB, a protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs
- Has no substantive effect on the risks to research participants
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s).

An unplanned protocol variance is considered a violation if the variance meets any of the following criteria:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

If a deviation or violation occurs please follow the guidelines below:

**Protocol Violations:** Violations should be reported by UNC personnel within one (1) week of the investigator becoming aware of the event using the same IRB online mechanism used to report Unanticipated Problems.

**Unanticipated Problems:**

UNC
Any events that meet the criteria for "Unanticipated Problems" as defined by UNC's IRB must be reported by the Study Coordinator using the IRB's web-based reporting system.

## 10.6 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator at UNC. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the subject, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to UNC's IRB for approval prior to implementation.

## 10.7 Record Retention

Study documentation includes all eCRFs, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed subject consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that all study documentation pertaining to the conduct of a clinical trial must be retained by the study investigator. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study

documents should be kept on file until three years after the completion and final study report of this investigational study.

### 10.8 Obligations of Investigators

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The Principal Investigator is responsible for personally overseeing the treatment of all study subjects. The Principal Investigator must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator will be responsible for assuring that all the required data will be collected and entered into the eCRFs. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all eCRFs will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

## 11.0 REFERENCES

1. Katz, A.J., et al., *Acute lymphoblastic leukemia: an assessment of international incidence, survival, and disease burden.* Cancer Causes Control, 2015.
2. Kaplan, J.B., M. Grischenko, and F.J. Giles, *Blinatumomab for the treatment of acute lymphoblastic leukemia.* Invest New Drugs, 2015.
3. Hoelzer, D., et al., *Improved outcome in adult B-cell acute lymphoblastic leukemia.* Blood, 1996. **87**(2): p. 495-508.
4. Kumar, P., et al., *Allogeneic hematopoietic stem cell transplantation in adult acute lymphocytic leukemia: impact of donor source on survival.* Biol Blood Marrow Transplant, 2008. **14**(12): p. 1394-400.
5. Pui, C.H., M.V. Relling, and J.R. Downing, *Acute lymphoblastic leukemia.* N Engl J Med, 2004. **350**(15): p. 1535-48.
6. Nguyen, K., et al., *Factors influencing survival after relapse from acute lymphoblastic leukemia: a Children's Oncology Group study.* Leukemia, 2008. **22**(12): p. 2142-50.
7. Pulte, D., et al., *Recent trends in survival of adult patients with acute leukemia: overall improvements, but persistent and partly increasing disparity in survival of patients from minority groups.* Haematologica, 2013. **98**(2): p. 222-9.
8. Pui, C.H. and W.E. Evans, *A 50-year journey to cure childhood acute lymphoblastic leukemia.* Semin Hematol, 2013. **50**(3): p. 185-96.
9. Gaynon, P.S., et al., *Survival after relapse in childhood acute lymphoblastic leukemia: impact of site and time to first relapse--the Children's Cancer Group Experience.* Cancer, 1998. **82**(7): p. 1387-95.
10. Bhojwani, D. and C.H. Pui, *Relapsed childhood acute lymphoblastic leukaemia.* Lancet Oncol, 2013. **14**(6): p. e205-17.
11. Gaynon, P.S., et al., *Bone marrow transplantation versus prolonged intensive chemotherapy for children with acute lymphoblastic leukemia and an initial bone*

*marrow relapse within 12 months of the completion of primary therapy: Children's Oncology Group study CCG-1941.* J Clin Oncol, 2006. **24**(19): p. 3150-6.
12. Maude, S.L., et al., *CD19-targeted chimeric antigen receptor T-cell therapy for acute lymphoblastic leukemia.* Blood, 2015. **125**(26): p. 4017-23.
13. Topp, M.S., et al., *Safety and activity of blinatumomab for adult patients with relapsed or refractory B-precursor acute lymphoblastic leukaemia: a multicentre, single-arm, phase 2 study.* Lancet Oncol, 2015. **16**(1): p. 57-66.
14. Scheuermann, R.H. and E. Racila, *CD19 antigen in leukemia and lymphoma diagnosis and immunotherapy.* Leuk Lymphoma, 1995. **18**(5-6): p. 385-97.
15. van der Stegen, S.J., M. Hamieh, and M. Sadelain, *The pharmacology of second-generation chimeric antigen receptors.* Nat Rev Drug Discov, 2015. **14**(7): p. 499-509.
16. Eshhar, Z., et al., *Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors.* Proc Natl Acad Sci U S A, 1993. **90**(2): p. 720-4.
17. Dotti, G., et al., *Design and development of therapies using chimeric antigen receptor-expressing T cells.* Immunol Rev, 2014. **257**(1): p. 107-26.
18. Jena, B., G. Dotti, and L.J. Cooper, *Redirecting T-cell specificity by introducing a tumor-specific chimeric antigen receptor.* Blood, 2010. **116**(7): p. 1035-44.
19. Sadelain, M., R. Brentjens, and I. Riviere, *The basic principles of chimeric antigen receptor design.* Cancer Discov, 2013. **3**(4): p. 388-98.
20. Imai, C., et al., *Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia.* Leukemia, 2004. **18**(4): p. 676-84.
21. Dotti, G., B. Savoldo, and M. Brenner, *Fifteen years of gene therapy based on chimeric antigen receptors: "are we nearly there yet?".* Hum Gene Ther, 2009. **20**(11): p. 1229-39.
22. Pule, M., H. Finney, and A. Lawson, *Artificial T-cell receptors.* Cytotherapy, 2003. **5**(3): p. 211-26.
23. Jensen, M.C., et al., *Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans.* Biol Blood Marrow Transplant, 2010. **16**(9): p. 1245-56.
24. Kochenderfer, J.N., et al., *B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells.* Blood, 2012. **119**(12): p. 2709-20.
25. Brentjens, R.J., et al., *Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias.* Blood, 2011. **118**(18): p. 4817-28.
26. Porter, D.L., et al., *Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia.* N Engl J Med, 2011. **365**(8): p. 725-33.
27. Brentjens, R.J., et al., *CD19-targeted T cells rapidly induce molecular remissions in adults with chemotherapy-refractory acute lymphoblastic leukemia.* Sci Transl Med, 2013. **5**(177): p. 177ra38.
28. Grupp, S.A., et al., *Chimeric antigen receptor-modified T cells for acute lymphoid leukemia.* N Engl J Med, 2013. **368**(16): p. 1509-18.
29. Maude, S.L., et al., *Chimeric antigen receptor T cells for sustained remissions in leukemia.* N Engl J Med, 2014. **371**(16): p. 1507-17.

30. Lee, D.W., et al., *T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial.* Lancet, 2015. **385**(9967): p. 517-28.
31. Davila, M.L., et al., *Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia.* Sci Transl Med, 2014. **6**(224): p. 224ra25.
32. Maus, M.V., et al., *Antibody-modified T cells: CARs take the front seat for hematologic malignancies.* Blood, 2014. **123**(17): p. 2625-35.
33. Lee, D.W., et al., *Current concepts in the diagnosis and management of cytokine release syndrome.* Blood, 2014. **124**(2): p. 188-95.
34. Frey, N.V. and D.L. Porter, *Cytokine release syndrome with novel therapeutics for acute lymphoblastic leukemia.* Hematology Am Soc Hematol Educ Program, 2016. **2016**(1): p. 567-572.
35. Porter, D.L., et al., *Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia.* Sci Transl Med, 2015. **7**(303): p. 303ra139.
36. Tey, S.K., et al., *Inducible caspase 9 suicide gene to improve the safety of allodepleted T cells after haploidentical stem cell transplantation.* Biol Blood Marrow Transplant, 2007. **13**(8): p. 913-24.
37. Di Stasi, A., et al., *Inducible apoptosis as a safety switch for adoptive cell therapy.* N Engl J Med, 2011. **365**(18): p. 1673-83.
38. Iuliucci, J.D., et al., *Intravenous safety and pharmacokinetics of a novel dimerizer drug, AP1903, in healthy volunteers.* J Clin Pharmacol, 2001. **41**(8): p. 870-9.
39. Turtle, C.J., et al., *CD19 CAR-T cells of defined CD4+:CD8+ composition in adult B cell ALL patients.* J Clin Invest, 2016. **126**(6): p. 2123-38.
40. Curran, K.J., H.J. Pegram, and R.J. Brentjens, *Chimeric antigen receptors for T cell immunotherapy: current understanding and future directions.* J Gene Med, 2012. **14**(6): p. 405-15.
41. Park, J.R., et al., *Adoptive transfer of chimeric antigen receptor re-directed cytolytic T lymphocyte clones in patients with neuroblastoma.* Mol Ther, 2007. **15**(4): p. 825-33.
42. Haji-Fatahaliha, M., et al., *CAR-modified T-cell therapy for cancer: an updated review.* Artif Cells Nanomed Biotechnol, 2016. **44**(6): p. 1339-49.
43. Gill, S. and C.H. June, *Going viral: chimeric antigen receptor T-cell therapy for hematological malignancies.* Immunol Rev, 2015. **263**(1): p. 68-89.
44. Grupp, S.A., et al., *Adoptive transfer of autologous T cells improves T-cell repertoire diversity and long-term B-cell function in pediatric patients with neuroblastoma.* Clin Cancer Res, 2012. **18**(24): p. 6732-41.
45. Vanin, E.F., et al., *Characterization of replication-competent retroviruses from nonhuman primates with virus-induced T-cell lymphomas and observations regarding the mechanism of oncogenesis.* J Virol, 1994. **68**(7): p. 4241-50.
46. Hacein-Bey-Abina, S., et al., *A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency.* N Engl J Med, 2003. **348**(3): p. 255-6.
47. Hacein-Bey-Abina, S., et al., *LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1.* Science, 2003. **302**(5644): p. 415-9.
48. Puck, J.M., et al., *Prenatal test for X-linked severe combined immunodeficiency by analysis of maternal X-chromosome inactivation and linkage analysis.* N Engl J Med, 1990. **322**(15): p. 1063-6.

49. Uribe, L. and K.I. Weinberg, *X-linked SCID and other defects of cytokine pathways.* Semin Hematol, 1998. **35**(4): p. 299-309.
50. Brenner, M.K., et al., *Gene marking to determine whether autologous marrow infusion restores long-term haemopoiesis in cancer patients.* Lancet, 1993. **342**(8880): p. 1134-7.
51. Heslop, H.E., et al., *Long-term restoration of immunity against Epstein-Barr virus infection by adoptive transfer of gene-modified virus-specific T lymphocytes.* Nat Med, 1996. **2**(5): p. 551-5.
52. Rooney, C.M., et al., *Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients.* Blood, 1998. **92**(5): p. 1549-55.
53. Heslop, H.E., et al., *Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients.* Blood, 2010. **115**(5): p. 925-35.
54. Essig, S., et al., *Risk of late effects of treatment in children newly diagnosed with standard-risk acute lymphoblastic leukaemia: a report from the Childhood Cancer Survivor Study cohort.* Lancet Oncol, 2014. **15**(8): p. 841-51.
55. Ghimire, K.B. and B.K. Shah, *Second primary malignancies in adult acute lymphoblastic leukemia: a US population-based study.* Blood, 2014. **124**(12): p. 2000-1.

# 12.0 APPENDICES

## 12.1 Appendix A: Performance Status (Lansky and Karnofsky)

| ***Performance Status Criteria***<br><br>***Karnofsky and Lansky performance scores are intended to be multiples of 10*** | |
|---|---|
| Karnofsky | |
| Score | Description |
| 100 | Normal, no complai |
| 90 | Able to carry on nor |
| 80 | Normal activity with |
| 70 | Cares for self, unabl |
| 60 | Required occasiona needs |
| 50 | Requires considerab |
| 40 | Disabled, requires s |
| 30 | Severely disabled, h |
| 20 | Very sick, hospitaliz |

| | |
|---|---|
| 10 | Moribund, fatal pro |

## 12.2 Appendix B: CRS Grading Criteria and Management Guidelines

**Version 2.0: August 2017**

### B A C K G R O U N D

Immunotherapies in cancer care are becoming more widely available. As these therapies are being used more commonly, clinicians must be aware of their unique toxicities and the optimal strategies that are recommended for the management of these toxicities. One toxicity in particular associated with these immunotherapies is cytokine release syndrome (CRS). This life-threatening toxicity, if not managed both appropriately and in a timely manner, can lead to multi-organ failure and death.

Cytokine release syndrome has been observed with several different immunotherapies, including monoclonal antibodies, bi-specific antibodies, T-cell checkpoint inhibitors, and novel T-cell therapies. It is characterized by widespread activation and proliferation of lymphocytes leading to an abundant release of inflammatory cytokines well above physiologic levels. This cytokine storm can manifest in many ways from constitutional symptoms to cardiovascular and neurological compromise. Management of this cytokine release storm involves both supportive care, and if clinically warranted, immunosuppression that blunts the aggressive cytokine response. However, administration of immunosuppressive therapies may also counter the desired immune response against targeted tumor cells. Thus, it is important that clinicians be prudent and reserve certain immunosuppressive strategies for the most appropriate clinical scenario. Thus, an algorithm that defines different grades of CRS and the corresponding therapy is necessary to guide clinicians in the delivery of appropriate care.

### S I G N S / S Y M P T O M S & C L I N I C A L G R A D I N G

Severity of cytokine release syndrome is variable and may be influenced by tumor burden at the time of treatment with the immune-directed therapy or other pre-existing comorbidities. Clinical grading is important for appropriate management. Organ systems affected by CRS and their corresponding signs and symptoms are listed below in Table 1 and criteria for clinical grading are outlined below in Table 2.

**TABLE 1. Signs and Symptoms of CRS**

| Organ system | Signs/Symptoms |
|---|---|
| Constitutional | Fever, rigors, malaise, fatigue, anorexia, myalgias/arthralgias, nausea/vomiting |
| Dermatologic | Rash |
| Gastrointestinal | Nausea/vomiting/diarrhea |
| Respiratory | Tachypnea, hypoxemia (potentially requiring supplemental oxygen/ventilation) |
| Cardiovascular | Tachycardia, hypotension |
| Coagulation | Disseminated intravascular coagulation (DIC) characterized by elevated D-dimer, hypofibrinogenemia, bleeding |
| Renal | Azotemia |
| Hepatic | Transaminitis, hyperbilirubinemia |
| Neurologic | Altered mental status, confusion, delirium, aphasia, hallucinations, tremor, seizures, ataxia |

**CRS Grading/Severity**

The outlined clinical grading criteria is designed to guide clinicians in the management of CRS. Many of the signs and symptoms associated with CRS can also be attributable to other common complications of cancer therapy such as neutropenic fever, other infectious complications, and tumor lysis syndrome. Thus, in applying the criteria below, clinicians should exercise appropriate clinical judgement in each patient-specific scenario in an effort to distinguish true CRS from other cancer treatment-related toxicities.

This will ensure appropriate delivery of care and avoidance of therapies that may otherwise not be indicated.

**TABLE 2. CRS Grading**

| **Grade 1 – Mild (Symptomatic Management)** |
|---|
| Symptoms largely limited to constitutional symptoms listed above (Table 1)<br>Only requires symptomatic management |
| **Grade 2 – Moderate (Moderate Intervention)** |
| Hypotension responsive to fluids or single, low dose vasopressor<br>Oxygen requirement <40%<br>Grade 2 organ toxicity |
| **Grade 3 – Severe (Aggressive Intervention)** |
| Hypotension requiring high dose or >1 vasopressor<br>Oxygen requirement ≥40%<br>Grade 3 organ toxicity<br>Grade 4 transaminitis |
| **Grade 4 – Life-threatening (Life-sustaining intervention)** |
| Ventilator support required<br>Grade 4 organ toxicity |

**MANAGEMENT OF CRS**

Management of CRS involves both supportive measures and pharmacologic therapies that inhibit immune activation and amplification. Supportive measures are directed at managing constitutional symptoms and achieving and maintaining hemodynamic stability. Immune targeted therapies are directed at the cytokines released in the pathobiology of the syndrome. Cytokines identified to play a role in CRS include, but are likely not limited to, TNFα, IFNy, IL-1β, IL-2, IL-5, IL-6, IL-8, and IL-10. Therapies utilized in CRS exhibit either a non-specific inhibition of immune amplification or a more targeted inhibition of a particular cytokine. These therapies are outlined below.

**Tocilizumab**

Tocilizumab is a humanized monoclonal IL-6 receptor antibody that inhibits IL-6 from binding to both cell-associated and soluble IL-6 receptors. IL-6 is a cytokine that has been implicated in the pathogenesis of CRS and presents a pharmacologic target for management. Thus, treatment strategies for severe CRS have focused on inhibiting IL-6 signaling. Tocilizumab can be administered in severe or life-threatening cases (Grade 3 or 4), resulting in a rapid reversal of symptoms. If there is lack of clinical improvement within 24 hours, a second dose can be repeated.

- Adults: 4 mg/kg IV over 1 hour for one dose. May repeat another dose at 8 mg/kg if lack of clinical response to initial 4 mg/kg within 24 hours.
- Pediatrics: 8 mg/kg IV over 1 hour for one dose. May repeat another 8 mg/kg dose if lack of clinical response to initial dose within 24 hours.

**Corticosteroids**

Steroids may be utilized as a 1st or 2nd line approach, or in a severe setting, along with targeted cytokine therapies (i.e. tocilizumab). Steroids exhibit a non-specific immune inhibition. Intravenous dexamethasone is the preferred steroid to be initiated if steroids are warranted (i.e. neurological symptoms) given improved CNS penetration. Methylprednisolone can be used as an alternative. With appropriate response, steroids can be tapered over several days (~1 week). Dosing is as follows.

- Dexamethasone;
    - Adult: 4-10 mg (max single dose 10 mg) IV q 6 hours
    - Pediatrics: 0.1 mg/kg (max single dose 10 mg) IV q 6 hours
- Methylprednisolone
    - Adults: 2 mg/kg/day IV; Tapered with clinical improvement
    - Pediatrics: 2 mg/kg/day IV; Tapered with clinical improvement; max daily dose 125 mg

# TREATMENT ALGORITHM

**Important Considerations**

- **The following adult and pediatric treatment algorithms serve as a framework for the management of patients with CRS and is not meant to replace physician discretion. Given that each patient will require thorough clinical evaluation for proper management, an attending physician should be notified at the first signs/symptoms suggestive of CRS and should be involved in each therapeutic decision made throughout the progression of care. This includes supportive and pharmacologic interventions, as well as escalation of care from the floor to ICU-level care.**

- **Investigational Protocol: Some patients may receive AP1903/Rimiducid on an investigational protocol for CRS instead of tocilizumab as outlined in the algorithms below. Enrollment on this investigational study should be verified before tocilizumab is ordered. For enrolled patients, the investigational protocol should be followed.**

- **Initiation of tocilizumab and/or steroids for CRS from CAR-T therapy must be approved by one of the following attendings in the link provided below:**
  **http://intranet.unchealthcare.org/intranet/hospitaldepartments/uncsct/cell_therapy_resources**

**Adult CRS Treatment Algorithm**

| Signs/Symptoms | Management |
|---|---|
| **Grade 1 CRS** | |
| - Fever (≥ $38.0^0$ C)<br>- +/- additional constitutional symptoms (rigors, malaise, fatigue, anorexia, myalgias/arthralgias, nausea/vomiting)<br>- **Renal:** Scr >ULN – 1.5 x ULN and/or Scr 1.5 – 2.0 x above baseline and/or Scr increase > 0.3 mg/dL from baseline<br>- **Hepatic:** AST/ALT > ULN – 2.5 x ULN T.bili > ULN – 1.5 x ULN | - Complete infectious workup (Cultures, Chest XR/CT, etc.)<br>- Daily weights; accurate I/Os<br>- Vital signs q30 mins. until symptom resolution<br>- **Initiate empiric antibiotics**<br>- **Supportive measures:** acetaminophen prn fevers; ondansetron or prochlorperazine prn nausea/vomiting; meperidine/morphine prn rigors |
| **Grade 2 CRS** | |
| - **CV:** Hypotension (SBP <90 mmHg or ≥ 20% decrease in baseline SBP or DBP) +/- asymptomatic tachycardia<br>- **Pulmonary:** Decreased O2 saturation (≤88%)<br>- **Renal:** Scr ≥ 1.5 – 3.0 x ULN and/or Scr 2.0 – 3.0 x above baseline<br>- **Hepatic:** AST/ALT > 2.5 – 5.0 x ULN and/or T. Bili > 1.5 – 3.0 x ULN | - Continue monitoring and supportive measures outlined under Grade 1 CRS<br>- **Supplemental O2 up to 40% to saturation ≥93%**<br>- **Fluid bolus: 1000 mL IV over 1-2 hours;**<br>- **Low dose vasopressor (Table 3) if unresponsive to fluid bolus** |
| **Grade 3 CRS** | |
| - **CV:** Hypotension (SBP <90 mmHg or ≥ 20% decrease in baseline SBP or DBP) unresponsive to fluids and low dose vasopressors given for grade 2 CRS (Table 3) +/- symptomatic tachycardia<br>- **Pulmonary:** Decreased O2 saturation (≤88%) requiring >40% to achieve saturation ≥93%<br>- **Neuro:** Neurologic symptoms (confusion, AMS, seizure) | - Continue monitoring and supportive measures outlined under Grade 1 and 2 CRS<br>- **Increase supplemental O2 to achieve saturation ≥93%**<br>- **High dose or multiple vasopressors (Table 3)**<br>- **Tocilizumab 4 mg/kg IV over 1 hour x 1 dose; may repeat with 8 mg/kg in 24 hours if no improvement** |

| | |
|---|---|
| - **Renal:** Scr > 3.0 – 6.0 x ULN and/or Scr > 3.0 x above baseline or > 4.0<br>- **Hepatic:** AST/ALT > 5.0 – 20.0 x ULN and/or T. Bili > 3.0 -10.0 x ULN | - **Dexamethasone 10 mg IV q6h if neurologic symptoms present** |
| **Grade 4 CRS** | |
| - **CV:** Persistent hemodynamic instability (hypotension and tachycardia refractory to aggressive fluids and pressor support)<br>- **Pulmonary:** Hypoxic respiratory failure: O2 saturation ≥93% not able to be achieved with supplemental O2 (nasal cannula, non-rebreather)<br>- **Neuro:** Neurologic symptoms (confusion, AMS, seizures)<br>- **Renal:** Scr > 6.0 x ULN<br>- **Hepatic:** AST/ALT > 20.0 x ULN and/or T. Bili > 10.0 x ULN | - Continue monitoring and supportive measures outlined in Grade 1, 2, and 3 CRS<br>- Continue/initiate vasopressors, tocilizumab, and dexamethasone as outlined in Grade 3 CRS<br>- **Ventilatory support**<br>- **Dialysis if indicated (CVVHD or HD)** |

**TABLE 3. High dose vasopressors in adult patients**

| Vasopressor | Dose (high dose)* |
|---|---|
| Norepinephrine | ≥ 20 mcg/min monotherapy |
| Dopamine | ≥ 10 mcg/kg/min |
| Phenylephrine monotherapy | ≥ 200 mcg/min |
| Epinephrine | ≥ 10 mcg/min |
| Vasopressin + additional vasopressor | Vasopressin + norepinephrine equivalent of ≥ 10 mcg/min |

*vasopressors at doses lower than those outlined are considered low dose vasopressors

**Pediatric CRS Treatment Algorithm**

| Signs/Symptoms | Management |
|---|---|
| **Grade 1 CRS** | |
| - Fever (≥ 38.0$^0$ C)<br>- +/- additional constitutional symptoms (rigors, malaise, fatigue, anorexia, myalgias/arthralgias, nausea/vomiting)<br>- **Renal:** Scr >ULN – 1.5 x ULN and/or Scr 1.5 – 2.0 x above baseline and/or Scr increase > 0.3 mg/dL from baseline<br>- **Hepatic:** AST/ALT > ULN – 2.5 x ULN and/or T.bili > ULN – 1.5 x ULN | - Complete infectious workup (Cultures, Chest XR/CT, etc.)<br>- Daily weights; accurate I/Os<br>- Vital signs q30 mins. until symptom resolution<br>- **Initiate empiric antibiotics**<br>- **Supportive measures:** acetaminophen prn fevers; ondansetron or prochlorperazine prn nausea/vomiting; meperidine/morphine prn rigors |
| **Grade 2 CRS** | |
| - **CV:** Hypotension +/- asymptomatic tachycardia (Tables 4 & 5)<br>- **Pulmonary:** Decreased O2 saturation (≤88%)<br>- **Renal:** Scr ≥ 1.5 – 3.0 x ULN and/or Scr 2.0 – 3.0 x above baseline<br>- **Hepatic:** AST/ALT > 2.5 – 5.0 x ULN and/or T. Bili > 1.5 – 3.0 x ULN | - Continue monitoring and supportive measures outlined under Grade 1 CRS<br>- **Supplemental O2 up to 40% to saturation ≥93%**<br>- **Fluid bolus: 20 mL/kg (Max: 1000 mL) IV over 1-2 hours;**<br>- **Low dose vasopressor (Table 6) if unresponsive to fluid bolus** |
| **Grade 3 CRS** | |
| - **CV:** Hypotension unresponsive to fluids and low dose vasopressors given for grade 2 CRS +/- symptomatic tachycardia (Tables 4 and 5)<br>- **Pulmonary:** Decreased O2 saturation (≤88%) requiring >40% to achieve saturation ≥93%<br>- **Neuro:** Neurologic symptoms (confusion, AMS, seizure)<br>- **Renal:** Scr > 3.0 – 6.0 x ULN and/or Scr > 3.0 x above baseline or > 4.0<br>- **Hepatic:** AST/ALT > 5.0 – 20.0 x ULN and/or T. Bili > 3.0 -10.0 x ULN | - Continue monitoring and supportive measures outlined under Grade 1 and 2 CRS<br>- **Increase supplemental O2 to achieve saturation ≥93%**<br>- **High dose or multiple vasopressors (Table 6)**<br>- **Tocilizumab 8 mg/kg IV over 1 hour x 1 dose; may repeat in 24 hours if no improvement**<br>- **Dexamethasone 0.1 mg/kg (Max 10 mg) IV q6h if neurologic symptoms present** |
| **Grade 4 CRS** | |
| - **CV:** Persistent hemodynamic instability (hypotension and tachycardia refractory to aggressive fluids and pressor support)<br>- **Pulmonary:** Hypoxic respiratory failure: O2 saturation ≥93% not able to be achieved with supplemental O2 (nasal cannula, non-rebreather)<br>- **Neuro:** Neurologic symptoms (confusion, AMS, seizures)<br>- **Renal:** Scr > 6.0 x ULN<br>- **Hepatic:** AST/ALT > 20.0 x ULN and/or T. Bili > 10.0 x ULN | - Continue monitoring and supportive measures outlined in Grade 1, 2, and 3 CRS<br>- Continue/initiate vasopressors, tocilizumab, and dexamethasone as outlined in Grade 3 CRS<br>- **Ventilatory support**<br>- **Dialysis if indicated (CVVHD or HD)** |

**TABLE 4. Definition of hypotension in pediatric patients**

| Age | Hypotension (SBP) |
|---|---|
| 0 – 28 days | < 60 mmHg |
| 1 – 12 months | < 70 mmHg |
| 1 – 10 years | < 70 mmHg + (age x 2) |
| >10 years | < 90 mmHg |

**TABLE 5. Definition of tachycardia in pediatric patients**

| Age | Tachycardia (HR at Rest) | Tachycardia (HR while Awake) |
|---|---|---|
| 0 – 3 months | > 160 BPM | > 205 BPM |
| 3 months – 2 years | > 160 BPM | > 190 BPM |
| 2 – 10 years | > 90 BPM | > 140 BPM |
| >10 years | > 90 BPM | > 100 BPM |

**TABLE 6. High dose vasopressors in pediatric patients**

| Vasopressor | Dose (high dose)* |
|---|---|
| Norepinephrine | ≥ 2 mcg/kg/min |
| Dopamine | ≥ 10 mcg/kg/min |
| Phenylephrine monotherapy | ≥ 5 mcg/kg/min |
| Epinephrine | ≥ 1 mcg/kg/min |
| Vasopressin | > 100 milliunits/kg/hr |

*vasopressors at doses lower than those outlined are considered low dose vasopressors

## References

1. Maude SL, Barrett D, Teachey DT, Grupp S. Managing cytokine release syndrome associated with novel T cell-engaging therapies. *J Cancer* 2014;20(2):119-122.
2. Lee DW, Gardner R, Porter DL, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood* 2014;124(2):188-195.
3. Frey NV, Levine BL, Lacey SF, et al. Refractory cytokine release syndrome in recipients of chimeric antigen receptor (CAR) T cells. *Blood* 2014;124(21):2296.
4. National Cancer Institute Common Terminology Criteria for Adverse Events v4.03; 2010. Available online at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html (accessed 11/28/2015).

## 12.3 Appendix C: Abbreviated Follow Up Required After Disease Progression

Subjects should not receive other antineoplastic agents for at least 4 weeks post T cell infusion (for purposes of evaluation). Subjects who experience disease progression after a cell infusion may receive other therapy if needed at the discretion of their attending physician. If subjects receive other therapy they will come off study for adverse event reporting after the 4-week assessments outlined in Section 7.1 are completed. Subsequent follow up assessments for subjects post progression are outlined in the table below. If subjects develop PD after they have completed the initial 4-week follow up period for the cell infusion, they should be contacted as outlined below. The timing for each visit relates to the last cell infusion received by the subject. Follow up kits and instructions will be provided to subjects who experience PD. Blood samples should be collected by a local health care provider as outlined in section 12.5.1.

| **Assessments**[1] | **Post Disease Progression Follow up** | | | | |
|---|---|---|---|---|---|
| | Week 8 D1 | Month 3 D1 | Month 6 D1 | Mth 12 D1 | Yearly |
| History[2] | X | X | X | X | X |
| Performance status | X | X | X | X | X |
| RCR by PCR and archive samples[3,5] | | X | X | X | X |
| Quantitative PCR[4,5] | X | X | X | X | X |
| Survival | X | X | X | X | X |

1. A window of +/-10 days will apply to the every 3 months study visits, and a window of +/-30 days will apply to visits separated by ≥ 6 months. Yearly follow-up visits are required during long-term follow-up for a total of 15 years or until death.
2. History and performance status information will be collected via the phone contact. Survival will also be documented.
3. RCR Testing by PCR: RCR testing will be done on samples collected at 8 weeks, 3 months, 6 months and 12 months after treatment. If all post treatment assays are negative during the first year, then further yearly samples should be archived. If any post treatment samples are positive, further analysis of the RCR and more extensive subject follow-up should be undertaken in consultation with the FDA.
4. Quantitative PCR to Test for Integrated Transgene: Quantitative real-time PCR to detect retroviral integrants will be collected 8 weeks post- T cell infusion and then at 3, 6, and 12 months then yearly for a total of 15 years. PCR to detect retroviral integrant clonality and integrant locus will also be done at the first occurrence (if the transgene is detected at >0.5%) and then every 3 months for the first year. If the transgene is still detected at > 0.5% level assays will continue every 6 months up to year 5. If the level of detection is less than 0.5% samples will be collected annually and may be archived. Follow up will continue for 15 years.
5. Collect (10ml) of blood into the 1 green top tube provided in the follow up kit for RCR by PCR and/or Quantitative PCR testing. See section 12.5.1 below for sample collection, handling and shipping instructions.

### 12.3.1 Follow up Kit Instructions

Thank you very much for your continuous participation in the long term safety monitoring of the CART study that you were enrolled in at the UNC. Please provide this handout to your healthcare provider.

At the scheduled doctor's visit, please have an appropriate staff member collect blood in the tube provided in the follow up kit.

The Follow up Kit Includes:

- 1 green top (10ml) tube included with a label that is pre-affixed to the tube.
- **FedEx label** for overnight shipping
- Packaging materials for shipping the blood sample back to UNC

(Please complete the information requested on the pre-affixed label on the tube)

- Study Number LCCC1541-ATL
- Subject ID Number
- Subject Initials
- Date of Specimen

Processing instructions:
Once the blood is collected, repack the tube as originally packaged. Do not spin and do not place the blood in a refrigerator or dry ice. The tube should be kept at ambient (ie, room temperature) temperature.

**Please ship the blood sample the same day it is collected**. The blood should be shipped for overnight delivery via FEDEX to the following address:

Shipping address:
Barbara Savoldo
UNC Lineberger Immunotherapy Program
The University of North Carolina at Chapel Hill
125 Mason Farm Road
Marsico Hall, Suite 5203
Chapel Hill, NC 27599
Phone 919 962 8414
email: bsavoldo@med.unc.edu

**Please notify Dr. Savoldo of the shipment on the day the sample is collected either by phone or email**.

## 12.4 Appendix D: Patient Reported Outcome Surveys

### Global Health Scale

**Please respond to each item by marking one box per row.**

| | | Excellent | Very good | Good | Fair | Poor |
|---|---|---|---|---|---|---|
| Global01 | In general, would you say your health is: ..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| Global02 | In general, would you say your quality of life is:..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| Global03 | In general, how would you rate your physical health? ..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| Global04 | In general, how would you rate your mental health, including your mood and your ability to think?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| Global05 | In general, how would you rate your satisfaction with your social activities and relationships? ..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| Global09 | In general, please rate how well you carry out your usual social activities and roles. (This includes activities at home, at work and in your community, and responsibilities as a parent, child, spouse, employee, friend, etc.)..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| | | **Completely** | **Mostly** | **Moderately** | **A little** | **Not at all** |
| Global06 | To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?..... | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |

**In the past 7 days…**

| | | Never | Rarely | Sometimes | Often | Always |
|---|---|---|---|---|---|---|
| Global10 | How often have you been bothered by emotional problems such as feeling anxious, depressed or irritable? ..... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

| | | None | Mild | Moderate | Severe | Very severe |
|---|---|---|---|---|---|---|
| Global08 | How would you rate your fatigue on average? .... | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Global07 | How would you rate your pain on average?..... | ☐ 0 No pain | ☐ 1 | ☐ 2 | ☐ 3 | ☐ 4 | ☐ 5 | ☐ 6 | ☐ 7 | ☐ 8 | ☐ 9 | ☐ 10 Worst imaginable pain |

## Physical Function – Short Form 20a

**Please respond to each item by marking one box per row.**

| | | Without any difficulty | With a little difficulty | With some difficulty | With much difficulty | Unable to do |
|---|---|---|---|---|---|---|
| PFA11 | Are you able to do chores such as vacuuming or yard work? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA12 | Are you able to push open a heavy door? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA16 | Are you able to dress yourself, including tying shoelaces and doing buttons? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA34 | Are you able to wash your back? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA38 | Are you able to dry your back with a towel? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA51 | Are you able to sit on the edge of a bed? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA55 | Are you able to wash and dry your body? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA56 | Are you able to get in and out of a car? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFB19 | Are you able to squeeze a new tube of toothpaste? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFB22 | Are you able to hold a plate full of food? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFB24 | Are you able to run a short distance, such as to catch a bus? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |

| | | Without any difficulty | With a little difficulty | With some difficulty | With much difficulty | Unable to do |
|---|---|---|---|---|---|---|
| PFB26 | Are you able to shampoo your hair? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFC45 | Are you able to get on and off the toilet? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFC46 | Are you able to transfer from a bed to a chair and back? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| | | **Not at all** | **Very little** | **Somewhat** | **Quite a lot** | **Cannot do** |
| PFA1 | Does your health now limit you in doing vigorous activities, such as running, lifting heavy objects, participating in strenuous sports? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA3 | Does your health now limit you in bending, kneeling, or stooping? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFA5 | Does your health now limit you in lifting or carrying groceries? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFC12 | Does your health now limit you in doing two hours of physical labor? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFC36 | Does your health now limit you in walking more than a mile? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |
| PFC37 | Does your health now limit you in climbing one flight of stairs? | ☐ 5 | ☐ 4 | ☐ 3 | ☐ 2 | ☐ 1 |

# NCI PRO-CTCAE™ ITEMS

**Item Library Version 1.0**

As individuals go through treatment for their cancer they sometimes experience different symptoms and side effects. For each question, please check or mark an ☒ in the one box that best describes your experiences over the past 7 days...

| | | | | | |
|---|---|---|---|---|---|
| 1. | In the last 7 days, what was the SEVERITY of your DECREASED APPETITE at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did DECREASED APPETITE INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 2. | In the last 7 days, how OFTEN did you have NAUSEA? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your NAUSEA at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |

| | | | | | |
|---|---|---|---|---|---|
| 3. | In the last 7 days, how OFTEN did you have VOMITING? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your VOMITING at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |

| | | | | | |
|---|---|---|---|---|---|
| 4. | In the last 7 days, what was the SEVERITY of your CONSTIPATION at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |

| | | | | | |
|---|---|---|---|---|---|
| 5. | In the last 7 days, how OFTEN did you have LOOSE OR WATERY STOOLS (DIARRHEA)? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |

| | | | | | |
|---|---|---|---|---|---|
| 6. | In the last 7 days, how OFTEN did you have PAIN IN THE ABDOMEN (BELLY AREA)? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your PAIN IN THE ABDOMEN (BELLY AREA) at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did PAIN IN THE ABDOMEN (BELLY AREA) INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 7. | In the last 7 days, what was the SEVERITY of your SHORTNESS OF BREATH at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did your SHORTNESS OF BREATH INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 8. | In the last 7 days, what was the SEVERITY of your COUGH at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did COUGH INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 9. | In the last 7 days, how OFTEN did you have ARM OR LEG SWELLING? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your ARM OR LEG SWELLING at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did ARM OR LEG SWELLING INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 10. | In the last 7 days, how OFTEN did you feel a POUNDING OR RACING HEARTBEAT (PALPITATIONS)? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your POUNDING OR RACING HEARTBEAT (PALPITATIONS) at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |

| | | | |
|---|---|---|---|
| 11. | In the last 7 days, did you have any RASH? | |
| | ○ Yes | ○ No |

| | | | | | |
|---|---|---|---|---|---|
| 12. | In the last 7 days, what was the SEVERITY of your NUMBNESS OR TINGLING IN YOUR HANDS OR FEET at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did NUMBNESS OR TINGLING IN YOUR HANDS OR FEET INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 13. | In the last 7 days, what was the SEVERITY of your DIZZINESS at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did DIZZINESS INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 14. | In the last 7 days, what was the SEVERITY of your BLURRY VISION at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did BLURRY VISION INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 15. | In the last 7 days, what was the SEVERITY of your PROBLEMS WITH CONCENTRATION at their WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did PROBLEMS WITH CONCENTRATION INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 16. | In the last 7 days, what was the SEVERITY of your PROBLEMS WITH MEMORY at their WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did PROBLEMS WITH MEMORY INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 17. | In the last 7 days, how OFTEN did you have PAIN? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your PAIN at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did PAIN INTEREFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 18. | In the last 7 days, how OFTEN did you have a HEADACHE? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your HEADACHE at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did your HEADACHE INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 19. | In the last 7 days, how OFTEN did you have ACHING MUSCLES? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your ACHING MUSCLES at their WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did ACHING MUSCLES INTEREFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 20. | In the last 7 days, how OFTEN did you have ACHING JOINTS (SUCH AS ELBOWS, KNEES, SHOULDERS)? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your ACHING JOINTS (SUCH AS ELBOWS, KNEES, SHOULDERS) at their WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did ACHING JOINTS (SUCH AS ELBOWS, KNEES, SHOULDERS) INTEREFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 21. | In the last 7 days, what was the SEVERITY of your INSOMNIA (INCLUDING DIFFICULTY FALLING ASLEEP, STAYING ASLEEP, OR WAKING UP EARLY) at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did INSOMNIA (INCLUDING DIFFICULTY FALLING ASLEEP, STAYING ASLEEP, OR WAKING UP EARLY) INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 22. | In the last 7 days, what was the SEVERITY of your FATIGUE, TIREDNESS, OR LACK OF ENERGY at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did FATIGUE, TIREDNESS, OR LACK OF ENERGY INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 23. | In the last 7 days, how OFTEN did you feel ANXIETY? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your ANXIETY at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did ANXIETY INTERFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | | | | |
|---|---|---|---|---|---|
| 24. | In the last 7 days, how OFTEN did you have SAD OR UNHAPPY FEELINGS? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your SAD OR UNHAPPY FEELINGS at their WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| | In the last 7 days, how much did SAD OR UNHAPPY FEELINGS INTEFERE with your usual or daily activities? | | | | |
| | ○ Not at all | ○ A little bit | ○ Somewhat | ○ Quite a bit | ○ Very much |

| | | |
|---|---|---|
| 25. | In the last 7 days, did you BRUISE EASILY (BLACK AND BLUE MARKS)? | |
| | ○ Yes | ○ No |

| | | | | | |
|---|---|---|---|---|---|
| 26. | In the last 7 days, how OFTEN did you have SHIVERING OR SHAKING CHILLS? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your SHIVERING OR SHAKING CHILLS at their WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |

| | | | | | |
|---|---|---|---|---|---|
| 27. | In the last 7 days, how OFTEN did you have UNEXPECTED OR EXCESSIVE SWEATING DURING THE DAY OR NIGHTIME (NOT RELATED TO HOT FLASHES)? | | | | |
| | ○ Never | ○ Rarely | ○ Occasionally | ○ Frequently | ○ Almost constantly |
| | In the last 7 days, what was the SEVERITY of your UNEXPECTED OR EXCESSIVE SWEATING DURING THE DAY OR NIGHTIME (NOT RELATED TO HOT FLASHES) at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |

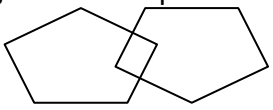
| | |
|---|---|
| Do you have any other symptoms that you wish to report? | |
| ○ Yes | ○ No |

Please list any other symptoms:

| | | | | | |
|---|---|---|---|---|---|
| 1. | In the last 7 days, what was the SEVERITY of this symptom at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| 2. | In the last 7 days, what was the SEVERITY of this symptom at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| 3. | In the last 7 days, what was the SEVERITY of this symptom at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| 4. | In the last 7 days, what was the SEVERITY of this symptom at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |
| 5. | In the last 7 days, what was the SEVERITY of this symptom at its WORST? | | | | |
| | ○ None | ○ Mild | ○ Moderate | ○ Severe | ○ Very severe |

## 12.5 Appendix E: Mini-Mental State Exam

***Instructions:** Ask the questions in the order listed. Score one point for each correct response within each question or activity.*

| Maximum Score | Patient's Score | Questions |
|---|---|---|
| 5 | | "What is the year? Season? Date? Day of the week? Month?" |
| 5 | | "Where are we now: State? County? Town/city? Hospital? Floor?" |
| 3 | | The examiner names three unrelated objects clearly and slowly, then asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible. Number of trials: _____ |
| 5 | | "I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, …) Stop after five answers.<br>Alternative: "Spell WORLD backwards." (D-L-R-O-W) |
| 3 | | "Earlier I told you the names of three things. Can you tell me what those were?" |
| 2 | | Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them. |
| 1 | | "Repeat the phrase: 'No ifs, ands, or buts.'" |
| 3 | | "Take the paper in your right hand, fold it in half, and put it on the floor."<br>(The examiner gives the patient a piece of blank paper.) |
| 1 | | "Please read this and do what it says." (Written instruction is "Close your eyes.") |
| 1 | | "Make up and write a sentence about anything." (This sentence must contain a noun and a verb.) |
| 1 | | "Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.)<br>  |
| 30 | | TOTAL |

(Adapted from Rovner & Folstein)

**Instructions for administration and scoring of the MMSE**

*Orientation (10 points):*

- Ask for the date. Then specifically ask for parts omitted (e.g., "Can you also tell me what season it is?"). One point for each correct answer.
- Ask in turn, "Can you tell me the name of this hospital (town, county, etc.)?" One point for each correct answer.

*Registration (3 points):*

- Say the names of three unrelated objects clearly and slowly, allowing approximately one second for each. After you have said all three, ask the patient to repeat them. The number of objects the patient names correctly upon the first repetition determines the score (0-3). If the patient does not repeat all three objects the first time, continue saying the names until the patient is able to repeat all three items, up to six trials. Record the number of trials it takes for the patient to learn the words. If the patient does not eventually learn all three, recall cannot be meaningfully tested.
- After completing this task, tell the patient, "Try to remember the words, as I will ask for them in a little while."

*Attention and Calculation (5 points):*

- Ask the patient to begin with 100 and count backward by sevens. Stop after five subtractions (93, 86, 79, 72, 65). Score the total number of correct answers.
- If the patient cannot or will not perform the subtraction task, ask the patient to spell the word "world" backwards. The score is the number of letters in correct order (e.g., dlrow=5, dlorw=3).

*Recall (3 points):*

- Ask the patient if he or she can recall the three words you previously asked him or her to remember. Score the total number of correct answers (0-3).

*Language and Praxis (9 points):*

- Naming: Show the patient a wrist watch and ask the patient what it is. Repeat with a pencil. Score one point for each correct naming (0-2).
- Repetition: Ask the patient to repeat the sentence after you ("No ifs, ands, or buts."). Allow only one trial. Score 0 or 1.
- 3-Stage Command: Give the patient a piece of blank paper and say, "Take this paper in your right hand, fold it in half, and put it on the floor." Score one point for each part of the command correctly executed.
- Reading: On a blank piece of paper print the sentence, "Close your eyes," in letters large enough for the patient to see clearly. Ask the patient to read the sentence and do what it says. Score one point only if the patient actually closes his or her eyes. This is not a test of memory, so you may prompt the patient to "do what it says" after the patient reads the sentence.

- Writing: Give the patient a blank piece of paper and ask him or her to write a sentence for you. Do not dictate a sentence; it should be written spontaneously. The sentence must contain a subject and a verb and make sense. Correct grammar and punctuation are not necessary.
- Copying: Show the patient the picture of two intersecting pentagons and ask the patient to copy the figure exactly as it is. All ten angles must be present and two must intersect to score one point. Ignore tremor and rotation.

## ***Sources:***

- Crum RM, Anthony JC, Bassett SS, Folstein MF. Population-based norms for the mini-mental state examination by age and educational level. *JAMA*. 1993;269(18):2386-2391.
- Folstein MF, Folstein SE, McHugh PR. "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res*. 1975;12:189-198.
- Rovner BW, Folstein MF. Mini-mental state exam in clinical practice. *Hosp Pract*. 1987;22(1A):99, 103, 106, 110.
- Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. *J Am Geriatr Soc*. 1992;40(9):922-935.