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Protocol PI	Loretta Nastoupil, MD
Department	Stem Cell Transplantation and Cellular Therapy
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Dose Escalation Study Phase I/II of Umbilical Cord Blood-Derived CAR-Engineered NK Cells in Conjunction with Lymphodepleting Chemotherapy in Patients with Relapsed/Refractory B-Lymphoid Malignancies

Institution Study Number: **2016-0641**

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Principal Investigator: Loretta Nastoupil, MD
The University of Texas MD Anderson Cancer Center
Stem Cell Transplantation and Cellular Therapy Department
1515 Holcombe Blvd, Unit 0432
Houston, TX 77030
Telephone: 713.792.2860
Fax: 713.794.5656
lnastoupil@mdanderson.org

Co-Chair: Chitra Hosing, MD[1]

Collaborators: Richard E. Champlin, MD[1]
Nitin Jain, MD[2]
Michael J. Keating, MD[2]
Partow Kebriaei, MD[1]
Sajad J. Khazal, MD[3]
Kris M. Mahadeo, MD[3]
Rohtesh Mehta, MD[1]
Yago Nieto, MD, PhD[1]
Amanda L. Olson, MD[1]
Betul Oran, MD[1]
Demetrios Petropoulos, MD[3]
Uday Popat, MD[1]
Muzaffar H. Qazilbash, MD[1]
Peter F. Thall, PhD[4]
Philip A. Thompson, MD[2] Michael Wang, MD[5]
William G. Wierda, MD[2]

[1] The University of Texas MD Anderson Cancer Center, Stem Cell Transplantation and Cellular Therapy Department
[2] The University of Texas MD Anderson Cancer Center, Leukemia Department
[3] The University of Texas MD Anderson Cancer Center, Division of Pediatrics
[4] The University of Texas MD Anderson Cancer Center, Biostatistics Department
[5] The University of Texas MD Anderson Cancer Center, Lymphoma/Myeloma Department

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1.0 Objectives

Primary objective:
To determine the safety and relative efficacy of CAR.CD19-CD28-zeta-2A-iCasp9-IL15-transduced CB-NK cells in patients with relapsed/refractory CD19+ B lymphoid malignancies.

Secondary Objectives:
1. To assess the overall response rate (complete and partial response rates).
2. To quantify persistence of infused allogeneic donor CAR-transduced CB-derived NK cells in the recipient.
3. To conduct comprehensive immune reconstitution studies.

2.0 Background

The aim of this clinical trial is to investigate novel immunotherapeutic strategies, using engineered natural killer (NK) cells to improve the tumor-free survival of patients with relapsed or refractory CD19+ B-cell malignancies. There are an annual average of 69,740 new cases of non-Hodgkin lymphoma (NHL), 15,680 new cases of chronic lymphocytic leukemia (CLL) and 6070 new cases of acute lymphoblastic leukemia (ALL) in the United States, with estimated annual death rates of 19,020, 4580 and 1,430 respectively (http:/www.cancer.org). Overall survival (OS) is determined largely by disease stage at presentation and response to chemotherapy. Standard therapy for patients who relapse following frontline therapy is allogeneic hematopoietic stem cell transplantation (HSCT). The expected OS for patients in 2nd complete remission is 25% based on chemotherapy-sensitivity at the time of HSCT. Thus, there is an urgent and unmet need to develop new therapies for patients with advanced B-lineage malignancies, especially because relapse after allogeneic HSCT is usually fatal.

Chronic lymphocytic leukemia (CLL) is the most common form of adult leukemia in the United States, accounting for 25% of all leukemias. There are more than 15,000 new cases of CLL and 4,500 deaths from CLL every year in the United States. The natural history of the disease is diverse. Patients with only lymphocytosis have a median survival greater than of 10 years, whereas those with evidence of marrow failure manifested by anemia or thrombocytopenia have a median survival of only 2-3 years. Since no treatment has been shown to be curative, nor is there objective evidence that a specific treatment prolongs survival, treatment is delayed.1The NCI-sponsored CLL Working Group proposed the following indications for initiating treatment: 1) weight loss of more than 10% over the preceding 6 months; 2) extreme fatigue attributable to progressive disease; 3) fever or night sweats without evidence of infection; 4) worsening anemia (Rai stage III) or thrombocytopenia (Rai stage IV); 5) massive lymphadenopathy (>10 cm) or rapidly progressive lymphocytosis (lymphocyte doubling time <6 months); or 6) prolymphocytic or Richter's transformation. Current treatments for newly diagnosed CLL include chemotherapy and antibody therapy either alone or in combination.[1] A variety of novel approaches such as targeted therapy using ibrutinib for treating CLL are being developed,[2;3] but the disease is not yet curable. Moreover, even after complete responses, immunological abnormalities and minimal residual disease remain in most patients. Ultimately, chronic immunosuppression resulting in infectious complications occurs in 80% of CLL patients and is a major cause of mortality. Allogeneic stem cell transplantation may be curative in some patients with CLL, but success has been limited, primarily due to the high

incidence of mortality and morbidity associated with the procedure.[4] Non-myeloablative allogeneic transplant regimens hold promise, but patient eligibility is limited by availability of matched sibling donors.

Historically the initial treatment of patients with CLL requiring treatment has been with an alkylating agent, particularly chlorambucil, alone or in combination with a corticosteroid. The overall response rate has been 50-70%; however, the complete remission rates are low (5-20%). Newer agents like purine analogues, particularly fludarabine, have higher response rates as initial treatment.[5] randomized trials comparing alkylating agent-based therapy with single-agent fludarabine have shown a higher complete response rate and longer disease-free survival with the nucleoside analogue, but have not shown a survival advantage.[5;6] Fludarabine was approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with B cell CLL who have not responded to or progressed during treatment with at least one alkylating agent-based regimen.

Combination regimens such as cyclophosphamide, fludarabine and Rituximab have been shown to improve response rates,[7] but these regimens are highly immunosuppressive, and long-term benefit has not been demonstrated. Ibrutinib is a covalent inhibitor of Bruton's tyrosine kinase (BTK), a member of the TEC tyrosine kinase family[8] and a key enzyme in the B-cell receptor signaling pathway. Ibrutinib as monotherapy, as well as in combination with immunotherapy or chemotherapy, is a very effective therapy for lymphoid malignancies including CLL.[2;9] However, outcomes after ibrutinib failure are dismal, with only a 3.1 month survival after drug discontinuation.[10]

Acute Lymphoblastic Leukemia. Allogeneic HCT is a curative approach for a select group of patients with ALL. Overall survival (OS) ranges from 30%-60% depending on the patients disease stage and risk profile at time of transplant.[11;12] Increasingly, minimal residual disease (MRD), both before and after HCT, is becoming an important predictor for relapse.[13] In a series of 149 ALL patients transplanted in remission at MD Anderson Cancer Center, patients with MRD, measured by multi-parameter flow cytometric immunophenotyping (FCI) with a sensitivity of 0.01%, present at time of HCT had a shorter PFS compared to patients who were MRD negative, 28% vs. 47%, p=.08 (4). Furthermore, among 135 patients who had MRD measured following HCT, 20 became positive for MRD, and 18 of these patients developed overt hematologic relapse within a median of 3.8 months.14 Of note, among 32 patients with overt relapse following HCT, 41% did NOT have preceding MRD, suggesting that positive MRD post HCT essentially confirms eventual relapse, but negative MRD post HCT in a high-risk patient does not preclude relapse. Our findings corroborate similar published studies.[15;16] Patients transplanted beyond second remission routinely have a significantly lower PFS and OS rates. In our study of 97 patients (CR1 51, CR2 29, others 17) treated with busulfan and clofarabine chemotherapy conditioning following a matched sibling (MSD) or matched unrelated donor (MUD) transplant, patients in CR1 had a significantly better disease free survival (DFS) compared with others. For patients in CR1, the 2-yr DFS rate was 61% with 9/51 patients relapsing at median 9 months, the 2-yr DFS rate was 40% for CR2, with 10/29 relapsing at median 3 months, and for patients with more advanced disease, the 2-yr DFS rate was 33% with 3/17 progressing at a median of 3 months. Data from the Center for International Blood and Marrow Transplant Research (CIBMTR) corroborate our findings. Between 1996 and 2001, in patients less than 20 years-old, OS ranges from 25% for patients transplanted beyond first remission to 50% for sibling transplants in first remission. Similarly, in adult patients, greater than 20 years-old, the best outcome is noted in sibling transplants done in first remission with OS of 60%, as compared to 35% if transplants are

performed beyond CR1 (CIBMTR Registry).
No effective treatment options exist for patients who relapse following HCT. Multiple published series report less than 10% survival for these patients, regardless of the treatment modality used, with a median survival of 2-3 months.[11-17] To date, the most common strategy employed to reduce relapse rates after HCT has usually involved some form of immune manipulation, ranging from donor lymphocyte infusion (DLI) to second transplant.[18-20] However, although it has been consistently shown that patients with B-ALL who develop graft-versus-host-disease (GVHD) have less risk for relapse, DLI has[2] not shown appreciable efficacy in this patient population; remission rates have been less than 10%, and have been associated with a high incidence of GVHD.[2] Of note, the best responses to DLI in ALL occur when the DLI is administered prophylactically to prevent relapse;[20] this approach has been demonstrated in pediatric patients but no data for prophylactic DLI has been reported in adults. Thus, there is an unmet need for effective therapy for ALL patients at high risk for relapse following allogeneic HCT, with high risk defined as positive MRD and/or disease beyond first complete remission.

Non-Hodgkins Lymphoma (NHL). In the United States, B cell lymphomas represent 80-85% of cases reported. In 2013 approximately 69,740 new cases of NHL and over 19,000 deaths related to the disease were estimated to occur. Non-Hodgkin lymphoma is the most prevalent hematological malignancy and is the seventh leading site of new cancers among men and women and account for 4% of all new cancer cases and 3% of deaths related to cancer (SEER 2014). Diffuse Large B cell Lymphoma: Diffuse large B cell lymphoma (DLBCL) is the most common subtype of NHL, accounting for approximately 30% of NHL cases. There are approximately 22,000 new diagnoses of DLBCL in the United States each year. First line therapy for DLBCL typically includes an anthracycline-containing regimen with rituximab. The first line objective response rate and the complete response (CR) rate to R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) is approximately 80% and 50% respectively. However, approximately one-third of patients have refractory disease to initial therapy or relapse after R-CHOP.[24] For those patients who relapse after response to first line therapy, approximately 40-60% of patients can achieve a second response with additional chemotherapy. For patients who are young and fit, the goal of second line therapy is to achieve a response that will make the patient eligible for autologous stem cell transplant (ASCT). The standard of care for second-line therapy for transplant-eligible patients includes rituximab and combination chemotherapy such as RICE (rituximab, ifosfamide, carboplatin, and etoposide) or RDHAP (rituximab, dexamethasone, cytarabine, and cisplatin). In a large randomized trial of RICE versus RDHAP in transplant-eligible patients with DLBCL (the CORAL study) 63% of patients achieved an objective response to either regimen with a 26% CR rate. Patients who respond to second line therapy and who are considered fit enough for transplant receive consolidation with high-dose chemotherapy and ASCT. This combination can cure approximately 50% of transplanted patients.[25] Patients who fail ASCT have a very poor prognosis and no curative options. The majority of second line patients are not eligible for ASCT due to chemotherapy-refractory disease, age, or comorbidities such as heart, lung, liver, or kidney disease. Transplant-ineligible salvage patients do not have a curative option available to them. There is no standard definition of relapse/refractory DLBCL. This trial will enroll patients with chemo-refractory lymphoma, as evidenced by failure to achieve even a transient or partial response to prior biologic and combination chemotherapy or by early recurrence after ASCT.

Transformed Follicular Lymphoma (TFL). Follicular lymphoma (FL), a B cell lymphoma, is the most common indolent (slow-growing) form of NHL, accounting for approximately 20% to 30%

of all NHLs. Some patients with FL will transform (TFL) histologically to DLBCL which is more aggressive and associated with a poor outcome. Histological transformation to DLBCL occurs at an annual rate of approximately 3% for 15 years with the risk of transformation continuing to drop in subsequent years. The biologic mechanism of histologic transformation is unknown. Initial treatment of TFL is influenced by prior therapies for follicular lymphoma but generally includes anthracycline-containing regimens with rituximab to eliminate the aggressive component of the disease (NCCN practice guidelines 2014). Treatment options for relapsed/refractory TFL are similar to those in DLBCL. Given the low prevalence of these diseases, no large prospective randomized studies in these patient populations have been conducted.[26] Patients with chemotherapy refractory disease have a similar or worse prognosis to those with refractory DLBCL.

In summary, subjects who have refractory, aggressive B lymphoid malignancies have a major unmet medical need and novel treatments are warranted in these populations.

NK cells:

Natural killer (NK) cells are an important component of the graft-versus-leukemia (GVL) response, which is critical to preventing relapse after HSCT.[33;34] Each mature NK cell expresses a wide array of activating and inhibitory killer immunoglobulin-like receptors (KIRs), which are specific for different HLA class-I molecules.[35-37] The ability of NK cells to recognize and kill malignant cells is governed by complex and poorly understood interactions between inhibitory signals resulting from the binding of inhibitory KIRs with their cognate HLA class-I ligands, and activating signals from activating receptors.[33;37;38] NK cell responses are mediated by two major effector functions: direct cytolysis of target cells and production of chemokines and cytokines. Through the latter mechanism (e.g., interferon-γ), NK cells participate in the shaping of the adaptive T cell response, possibly by a direct interaction between naïve T cells and NK cells migrating to secondary lymphoid compartments from inflamed peripheral tissues and by an indirect effect on dendritic cells (DC).[39;40]

GMP-grade NK cell expansion from cord blood. Previous studies have largely used freshly obtained peripheral blood NK cells. The low number of circulating peripheral blood NK cells severely limits their therapeutic utility. We have developed a system for ex vivo expansion of NK cells from cord blood (CB), which reliably generates clinically relevant doses of GMP grade CB-NK cells for adoptive immunotherapy, using GMP-grade K562-based artificial antigen-presenting cells (aAPCs) expressing membrane bound IL-21, 4-1BB ligand, CD64 (FcγRI) and CD86 (clone 9.mbIL21).[41] Cord blood is a novel, attractive source of NK cells for cellular immune therapy. The cells are already collected, stored and immediately available. The cord blood donor can be optimally selected for HLA type, KIR gene expression and other factors. The methodology to generate CB NK cells has been approved by the FDA.[42] Our current protocol yields a mean NK expansion of 3127 fold (range, 1640 – 4931 fold) (**Fig 1A**), with very few CD3+ cells (mean, 4.50 x 10^6) (**Fig 1B**).

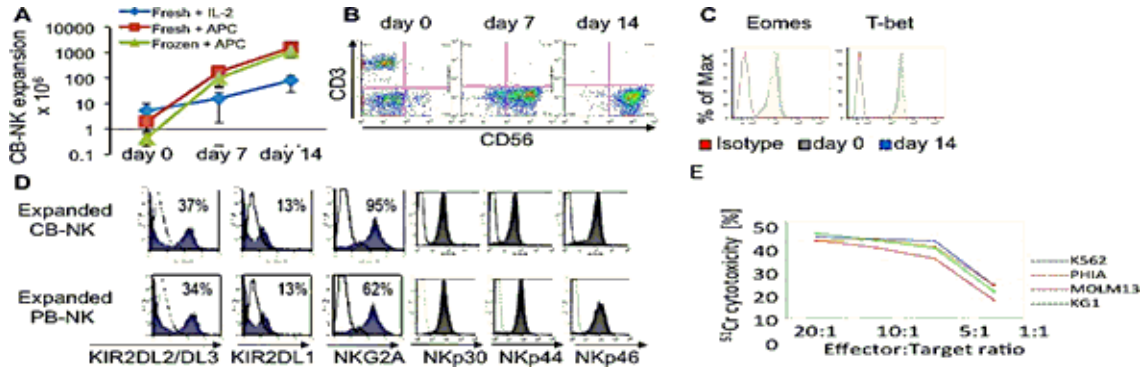

Fig 1. **Expansion, phenotype and anti-leukemia activity of ex vivo expanded CB-NK cells in vitro. A.** Culture of CB-NK cells**:** unselected fresh or frozen CB-MNCs were cultured for 7 days in a GP500 bioreactor with IL-2 alone or with IL-2 and clone 9.mbIL21 (antigen presenting cell, APC). Cells were immunomagnetically CD3-depleted on day 7 and re-cultured in the same conditions for an additional 7 days. On day 7, cells were again CD3-depleted and subjected to phenotypic and functional studies. **B.** Purity of NK product during expansion**. C.** APC-expanded CB-NK ells maintained Eomeshi and T-bethi phenotype. **D.** Representative histograms of NK receptor expression on ex-vivo expanded CB vs. PB NK cells. **E.** APC-expanded CB-NK cells demonstrate robust cytotoxicity against AML cell targets.

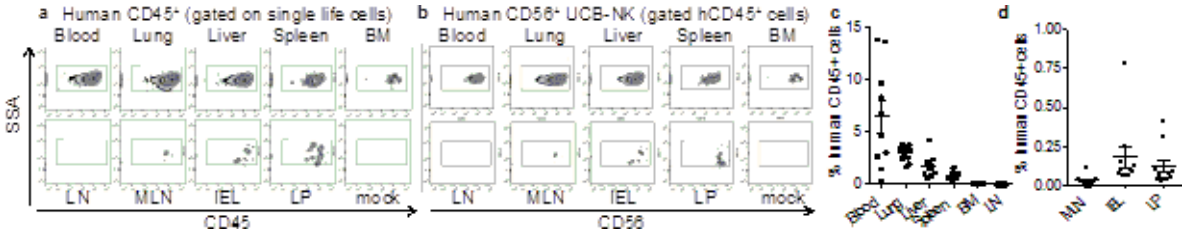

Fig. 2. Adoptively transferred CB-NK cells home to lymphoid and non-lymphoid tissues in NOD/SCID/IL2Rγc (NSG) mice. Intravenous infusion of 10 million CB-NK cells into lymphocyte-deficient NSG mice leads to homing of human NK cells to multiple lymphoid and non-lymphoid organs. (**A, B**) Flow cytometric analysis of single cell suspensions (live cells) for the detection of CB-NK cells. (**A**) Human CD45+ and (**B**) human CD56+ cells, gated on human CD45+ cells shown in (a). Ten recipients were analyzed at 20 hours after adoptive transfer. (**C, D**) Percentages of human CD45+CD56+ cells of total life single cells of each indicated organ.

Functional phenotype of ex vivo-expanded CB-NK cells and their cytotoxic activity against myeloid leukemia targets. The expanded CB-NK cells display the full array of activating and inhibitory receptors, continue to strongly express eomesodermin (Eomes) and T-bet (Fig 1C-D), two factors necessary for NK cell maturation and activation,[43;44] lyze myeloid target cells in a dose-dependent manner **(FIG. 1E)** and upon adoptive transfer into non-obese diabetic severe combined immunodeficient-gamma null (NSG) mice, could home to the bone marrow, liver, spleen and multiple lymphoid tissues **(FIG. 2)**.

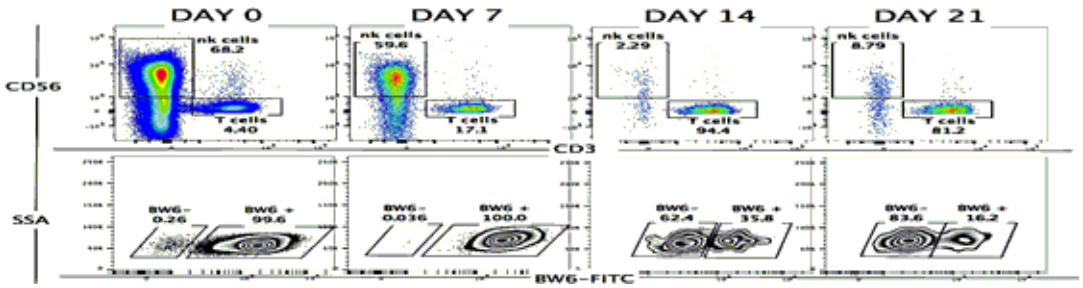

Fig. 3. Flow cytometric analysis to determine the persistence and phenotype of adoptively infused CB-NK cells in a patient with myeloma following high dose chemotherapy and autologous stem cell rescue. Using flow cytometry and HLA class I-specific antibodies the source of recovering NK cells (donor vs. recipient) is identified. In this case, the donor is HLA-Bw6+ve while the recipient is HLA-Bw6 negative. CB-derived NK cells are detectable in the peripheral blood of the recipient up to 21 days post-infusion.

MD Anderson experience with infusion of 'off-the-shelf' ex vivo expanded CB-derived NK cells. At MD Anderson Cancer Center, we have initiated a phase 1, first-in-human clinical trial with off-the-shelf allogeneic CB-NK cells in combination with high-dose chemotherapy and autologous hematopoietic stem cell transplant in patients with multiple myeloma (NCT01729091). To date we have treated 32 patients in three cohorts: 3 received 5 x10e6 expanded CB-NK cells/kg, 3 received 1 x10e7 CB-NK cells/kg, 3 received 5 x10e7 CB-NK cells/kg and 23 have received 1 x10e7 CB-NK cells/kg. Thus far the infusions of CB-NK cells appear to be well-tolerated without any toxicity. With follow-up times ranging from 1-24 months, there have been no cases of delayed recovery of neutrophil or platelet counts or GVHD. In the third and fourth cohorts we have detected CB-derived NK cells up to three weeks post-infusion (**Fig. 3**).

In a second phase I study (NCT01823198), we treated 18 patients who received HLA-compatible related or unrelated donor HSCT with increasing doses of ex vivo expanded NK cells (1 x10e6-1x10e8/kg) from third party (haploidentical relative or unrelated CB unit) to improve transplant outcomes. To date, there have been no major toxicities, no cases of graft failure and no increase in the incidence of GVHD. These studies confirm the safety and feasibility using ex vivo expanded CB-NK cells as a source of off-the-shelf immunotherapy.

Genetic modification of CB-derived NK cells to enhance their activity against leukemia. Chimeric antigen receptors (CARs) have been used extensively to redirect the specificity of T cells against leukemia[45-47] with dramatic clinical responses in patients with acute lymphoblastic leukemia (ALL).[48-50] These infusions have been primarily restricted to the autologous setting because activated T cells from an allogeneic source are likely to increase the risk of GVHD. Here, we propose to test the safety and efficacy of engineered CB-derived NK cells, as an alternative to T cells, for the immunotherapy of B-lymphoid malignancies. *CB-derived NK cells have multiple potential advantages over T cells*: (i) allogeneic NK cells should not cause GVHD, as predicted by observations in murine models, as well as patients with leukemia and solid malignancies treated with haploidentical or CB-derived NK cells;[51-53] (ii) mature NK cells have a limited life-span of a few weeks, allowing for antitumor activity while reducing the probability of long-term adverse events such as prolonged cytopenias caused by on-target/off-tumor toxicity to normal tissues, or the risk of malignant transformation; (iii) Unlike T-cells, NK cells will also have activity through their native receptors to kill antigen-negative target cells, potentially preventing a mechanism of immune escape; (iv) the generation of an autologous T cell product for each patient is logistically cumbersome and restrictive.[33;52] *The use of frozen, off-the-shelf*

CB units stored in the large global cord blood bank inventory for the generation of NK cells has the potential for widespread scalability that would not be possible with autologous peripheral blood-derived T or NK cell products.

Thus, to improve the persistence and anti-leukemic potency of frozen and ex vivo expanded CB-NK cells, we genetically modified them with a retroviral vector, iC9.CAR19-CD28-zeta-2A-IL15 (iC9/CAR.19/IL15), that (i) incorporates the gene for CAR-CD19 to redirect their specificity to CD19; (ii) ectopically produces IL-15, a cytokine crucial for NK cell survival and proliferation,[54-55] and (iii) expresses a suicide gene, based on inducible caspase-9 (iC9), that can be pharmacologically activated to eliminate transgenic cells as needed. Our preliminary data show that CB-NK cells can be stably transduced to express the CAR molecule (**Fig 4A**). Using a standard 51Cr-release assay, we found that iC9/CAR.19/IL15-transduced CB-NK cells had specific cytotoxic activity against CD19+ Raji cells and primary CLL cells (n=18; **Fig 4B**). The NK-CAR and non-transduced NK cells showed equal effector function against K562 cells, indicating that the genetic modification of CB-NK cells did not alter their intrinsic cytotoxicity against NK-sensitive targets **(Fig 4B**).

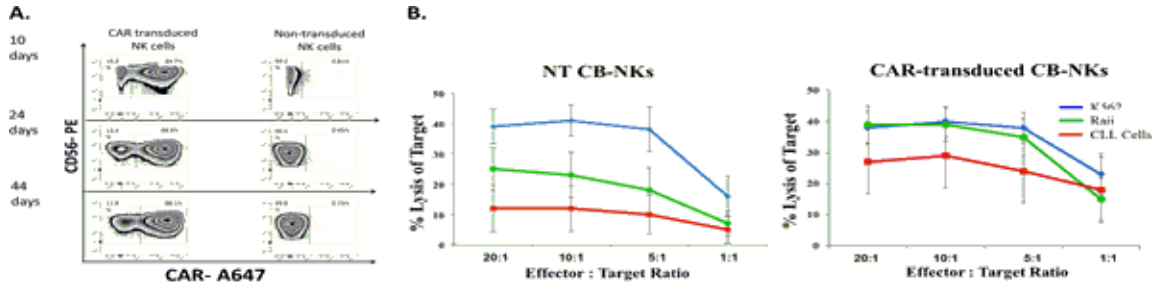

Fig. 4. Antileukemic function of CB-NK cells transduced with CAR19-CD28-zeta-2A-IL15 vector. (**A**) Transduction efficiency (85%) of CB NK cells (bottom panel) compared to non-transduced NK cells (top panel). Transduction is stable. (**B**) CAR-NK cells are more efficient at killing CD19+ Raji tumors and primary CLL compared to non-transduced (NT) ex vivo expanded and activated NK cells with equal effector function against K562 cells. P < 0.001 (iC9/CAR.CD19/IL15 + Raji vs NT-NKs + Raji); P < 0.001 (iC9/CAR.CD19/IL15 + CLL vs NT-NKs + CLL); P=ns (iC9/CAR.CD19/IL15 + K562 vs NT-NKs + K562).

We next evaluated the trafficking and persistence of iC9/CAR.19/IL15-modified CB-NK cells in vivo, using a NSG mouse Raji xenograft model. NT and iC9/CAR.19/IL15-transduced CB-NK cells were infused in mice engrafted with Raji cells. As shown in **Fig. 5A,** iC9/CAR.19/IL15+ CB-NK cells homed to the spleen, liver and bone marrow (sites of tumor infiltration), while CAR.CD19+ CB-NK cells without the IL-15 gene in the construct, as well as NT CB-NK cells were barely detectable in the tumor sites.

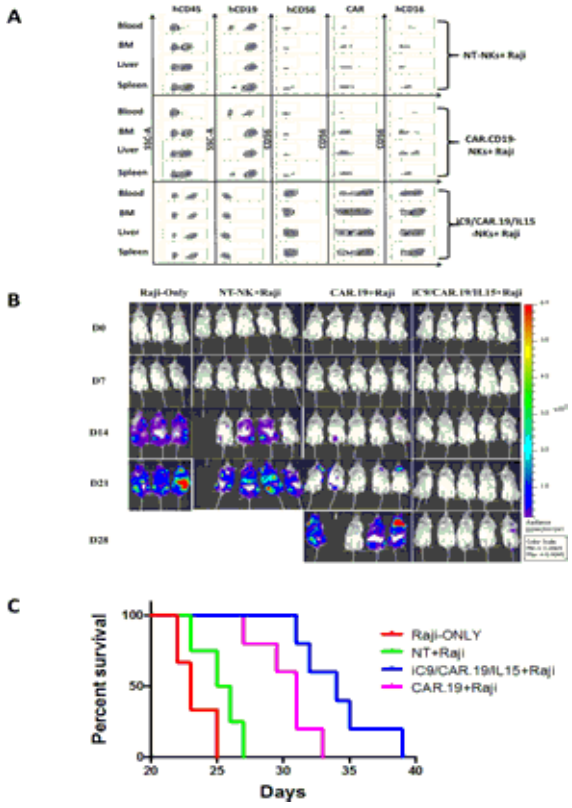

Fig. 5. In vivo homing, proliferation and antitumor activity of iC9/CAR.19/IL15-transduced CB NK cells. (**A**) iC9/CAR.19/IL15-tranduced eGFP-FFLuc-labeled CB-NK cells home to sites of disease (liver, spleen, bone marrow [BM]) more efficiently than CAR.19 transduced CB-NK cells or NT-NK cells. (**B**) Infusion of iC9/CAR.19/IL15-transduced CB-NK cells into NSG mice engrafted with luciferase-labeled Raji cells results in tumor eradication, as evidenced by in vivo bioluminescence imaging. Colors indicate intensity of luminescence (red, highest; blue, lowest). (**C**). The in vivo antitumor activity of a single dose of iC9/CAR.19/IL15-transduced CB NK is significantly better than that of CB-NK cells that were either not transduced or transduced with a CAR.CD19 construct lacking IL-15. P= 0.001 (iC9/CAR.CD19/IL15 + Raji vs NT-NKs + Raji); P= 0.044 (iC9/CAR.CD19/IL15 + Raji vs CAR.CD19 + Raji); P= 0.006 (CAR.CD19 + Raji vs NT-NKs + Raji) P= 0.182 (NT-NKs + Raji vs Raji alone).

iC9/CAR.19/IL15-tranduced CB-NK cells exert enhanced anti-tumor activity in vivo.
To study the in vivo antitumor activity of iC9/CAR.19/IL15-transduced CB-NK cells, we injected NSG mice with FFLuc-labeled Raji cells at 2 × 105/mouse. On the same day, mice received one i.v infusion of control NT, CAR.19 or iC9/CAR.19/IL15-transduced CB-NK cells (10 x 1^6/mouse). Tumor growth was monitored by measuring changes in tumor bioluminescence over time. As shown in **Fig. 5B**, tumor bioluminescence increased rapidly in mice engrafted with Raji cells and treated with control NT CB-NK cells. By contrast, infusion of either CAR.19+ or iC9/CAR.19/IL15+ CB-NK cells resulted in significant prolongation of survival compared to the effect of NT CB-NK cells (P=0.006 and P=0.001, respectively). Notably, iC9/CAR.19/IL15+ CB-NK cells controlled tumor expansion and prolonged survival (**Fig. 5C**) significantly better than the CAR.CD19 construct lacking the IL-15 gene, underscoring the important contribution of IL-15 to enhanced antitumor activity.

iC9/CAR.19/IL15-transduced CB-NK cells do not show in vitro or in vivo signs of autonomous or dysregulated growth. To investigate the possibility that the IL-15 gene in the vector may result in autonomous or dysregulated growth of transduced CB-NK cells, we cultured iC9/CAR.19/IL15-transduced CB-NK cells in complete Serum-free Stem Cell Growth Medium (SCGM) without the addition of exogenous IL-2 or clone 9.mbIL21 stimulation for 42 days (n=5). Viable cells were enumerated and passaged every three days by replacing media with fresh complete SCGM. As shown in **Fig 6A**, the iC9/CAR.19/IL15-transduced CB-NK cell cultures did not show any signs of abnormal growth over 6 weeks, after which, the cells stopped expanding. Karyotyping performed on iC9/CAR.19/IL15-transduced CB NK cells cultured for up to 17 weeks (n=7) failed to detect any chromosomal alterations (data not shown). We also performed chromosome and SNP microarray analyses on paired CB-NK cells (n=6) before (at baseline) and up to 22 weeks after CAR-transduction and ex vivo expansion, and did not observe any evidence of genetic instability (data not shown). With a follow-up exceeding 10 months, we did not observe any evidence of autonomous growth or leukemic transformation in mice treated with iC9/CAR.19/IL15 or CAR.19-transduced CB-NK cells. Histopathologic examination did not reveal any lymphocytic infiltration, proliferation or lymphoma in any tissue of these mice. The rudimentary lymphoid tissues of the spleen and lymph nodes were free of lymphocytes in all NSG mice from both groups of animals (**Fig 6B)**, nor was there any lymphocytic infiltration or proliferation in the bone marrow of these mice. Hematologic tests indicated normal numbers of white blood cells and lymphocytes, and no evidence of lymphocytic leukemia in both groups of mice.

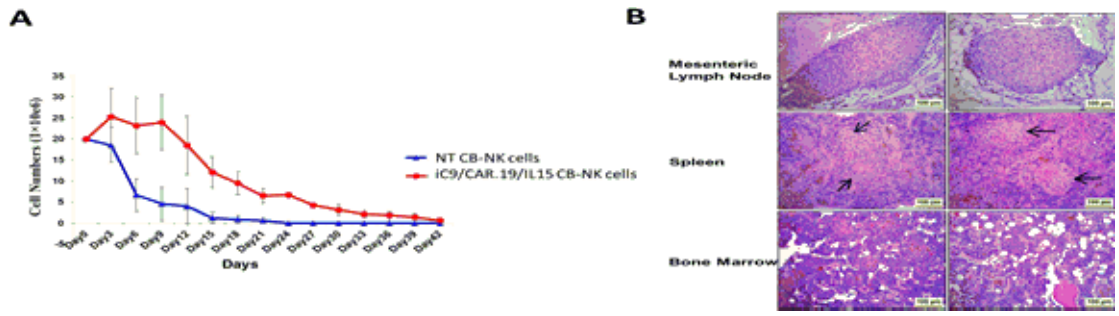

Fig. 6. IL-15-transduced CB-NK cells do not show signs of autonomous or dysregulated growth. (A) iC9/CAR.19/IL15-transduced CB NK cells stop expanding within 6 weeks of in vitro culture with no evidence of autonomous growth. **(B)** Photomicrographs of mesenteric lymph nodes show vestigial lymphoid tissue with no lymphocytes in any experimental mice, which is typical of NSG mice. Images of the spleen show rudimentary periarteriolar lymphoid tissue devoid of lymphocytes (black arrows) and is surrounded by hematopoietic tissue composed of various stages of erythroid and myeloid series cells, including megakaryocytes and hemosiderin-laden macrophages. Bone marrow contains normal hematopoietic cells and no abnormal lymphocytes. H&E stain, magnification x200. Slides from two representative groups of NSG mice treated with iC9/CAR.19/IL15-transduced CB NK cells.

IL-15 production by CAR-transduced NK cells

To verify that iC9/CAR.19/IL15+ CB-NK cells can produce IL-15, we cultured control NT CB-NK and iC9/CAR.19/IL15+ CB-NK lymphocytes in triplicates in the presence or absence of CD19+ CLL B cells and collected culture supernatants to measure IL15 release after 24, 48 and 72 hours of culture. As shown in **Fig. 7**, IL15 was undetectable in supernatants collected from non-transduced CB-NK cells cultured alone or with CLL targets. By contrast, iC9/CAR.19/IL15+ CB-NK cells produced small amounts of IL15 in

the absence of antigen stimulation [average 15.05 pg/mL/106 cells (range 6.2 – 23.47pg/mL)], which significantly increased with antigen stimulation [average 27.61 pg/mL/106 cells (range 15.82 – 38.18 pg/mL)] (P=0.02). We next examined the ability of iC9/CAR.19/IL15-transduced NK cells to produce IL-15 in vivo in NSG mice engrafted with Raji cells. Serum levels of IL-15 levels the height of NK cell expansion (2 weeks post expansion) were 40-50 pg/mL, and equivalent to levels detected in the supernatant of cultured cells.

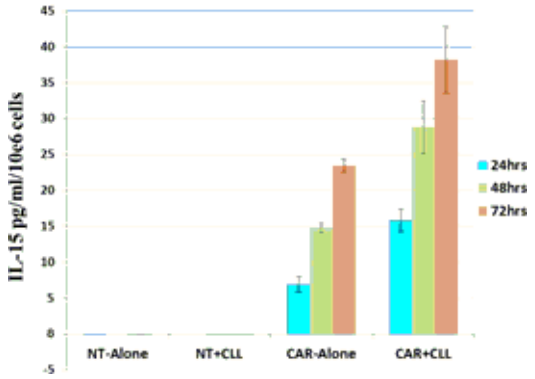

Fig. 7. IL-15 production and phenotype of iC9.CAR.19.CD28.CD3ζ.IL15-transduced CB NK cells. (A) iC9. CAR.19.CD28.CD3ζ.IL15-transduced CB NK cells produce IL-15 in response to antigenic stimulation in vitro

Exogenous recombinant human IL-15 (RhIL-15) has been used in the clinical setting. In a recent phase 1 study in patients with metastatic melanoma or renal cell carcinoma, bolus infusions of 3.0, 1.0, and 0.3 μg/kg per day of IL-15 were administered for 12 consecutive days to patients with metastatic malignant melanoma or metastatic renal cell cancer.[57] RhIL-15 was shown to activate NK cells, monocytes, γδ, and CD8 T cells. The 3.0-, 1.0-, and 0.3-μg/kg per day doses resulted in a maximum serum concentration (Cmax) of 43,800 ± 18,300, 15,900 ± 1,900, and 1,260 ± 350 pg/mL, respectively. Dose-limiting toxicities observed in patients receiving 3.0 and 1.0 μg/kg per day were grade 3 hypotension, thrombocytopenia, and elevations of ALT and AST, resulting in 0.3 μg/kg per day being determined the maximum-tolerated dose We do not anticipate severe toxicity releated to IL-15 release by iC9/CAR.19/IL15-transduced CB-NK cells, as the levels of IL-15 produced by the transduced NK cells are on average 2-3 logs lower than that achieved in the clinical trial of exogenous IL-15 treatment.[57]

iC9/CAR.19/IL15+ CB-NK cells are eliminated after activation of the suicide gene by exposure to a small-molecule dimerizer. To counteract the possibility of excessive toxicity mediated by the release of inflammatory cytokines by transduced CB-NK cells or uncontrolled NK cell growth, we incorporated a suicide gene based on the inducible caspase-9 gene in our construct.[56] As shown in **Fig. 8A,** the addition of as little as 10 nM of a small molecule dimerizer to cultures of iC9/CAR.19/IL15-transduced CB-NK cells induced apoptosis/necrosis of 60% of transgenic cells within 4 hours as assessed by annexin-V and 7AAD staining but had no effect on the viability of NT CB-NK cells. The suicide gene was also effective *in vivo*. Mice were engrafted i.v. with Raji tumor cells and treated with iC9/CAR.19/IL15-transduced CB-NK cells. Administration of the small-molecule dimerizer AP1903 (50 μg, i.p. 2 days apart) 10-14 days after NK cells had localized and expanded at different tumor sites later (**Fig. 8B,** left panel), resulted in a striking reduction in iC9/CAR.19/IL15-transduced CB-NK cells in the

blood and tissues of the treated mice (**Fig. 8B,** right panel), indicative of in vivo elimination of the transgenic cells.

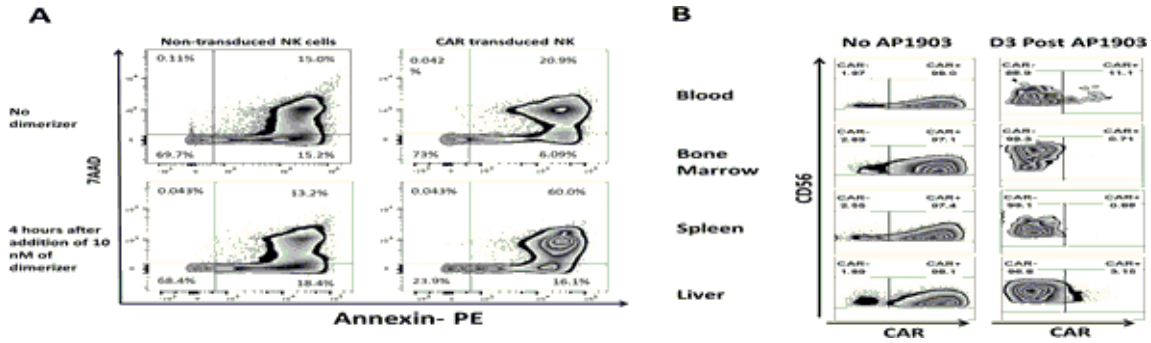

Fig. 8. Activation of the inducible caspase-9 suicide gene eliminates iC9/CAR.19/IL15+ CB-NK cells. (**A**) The addition of 10 nM of AP1903 to cultures of iC9-CAR-IL15+ CB-NK cells induced apoptosis/necrosis of transgenic cells (bottom right panel) within 4 hours as assessed by annexin-V-7AAD staining. NT, non-transduced CB-NK cells; CAR, iC9/CAR.19/IL15-transduced NK cells; (**B)** NSG mice engrafted i.v. with Raji cells, and infused with iC9/CAR.19/IL15+ CB-NK cells were treated 10-14 days later with two doses of the AP1903 dimerizer (50 µg) i.p. two days apart. iC9/CAR.19/IL15-expressing NK cells were substantially reduced in all organs tested 3 days later.

Clinical trial to evaluate the safety and efficacy of CB-NK cells transduced with iC9/CAR.19/IL15 in patients with relapsed/refractory B-lymphoid malignancies.

This is a Phase I/II dose-escalation trial to evaluate the safety and relative efficacy of iC9/CAR.19/IL15-transduced CB-NK cells in patients with relapsed/refractory B-lymphoid malignancies (ALL, CLL, NHL). This clinical study will capitalize on the synergistic antitumor activity produced by CAR CB-NK cells and the favorable lymphopenic environment induced by our lymphodepleting regimen.[58,59] Thus, patients will be treated with cyclophosphamide at a dose of 300 mg/m^2 /day for 3 days. Escalating doses of iC9/CAR.19/IL15-transduced CB-NK cells (10^5/kg-10^7/kg) will be infused once, on day 0, to determine the highest dose at which iC9/CAR.19/IL15-transduced CB-NK cells can be safely infused into patients with relapsed/refractory B-lymphoid malignancies, as defined by standard NCI toxicity criteria. A CB unit matched at 4/6, 5/6, or 6/6 HLA class I (serological) and II (molecular) antigens with the patient will be used for CB-NK expansion and CAR transduction. The CB units will be obtained from the MD Anderson cord blood bank.

To gain insight into the persistence, functionality and antileukemic potential of adoptively transferred iC9/CAR.19/IL15-transduced CB-NK cells, we have planned a series of phenotypic and functional assays. We will evaluate the magnitude of expansion and duration of persistence for adoptively infused genetically-modified NK cells in serially acquired PB samples by Q-PCR, using a primer pair that specifically amplifies the unique CAR transgene with sensitivity to detect 1/10,000 CAR+ NK cells. If there are sufficient numbers of circulating NK cells we will quantify by flow cytometry using a mAb specific against the CH2-CH3 region of iC9/CAR.19/IL15 with sensitivity to detect 1/1,000 CAR+ NK cells. The flow cytometry measurements will be coupled with analysis of cell surface NK activating and inhibitory receptor expression. We will evaluate for maintenance of CD19-redirected effector function, using 51Cr release assay, CD107a degranulation,[60,61] cytokine release (determined by intracellular cytokine assay for IFNγ and IL-

2) and chemokine release (MIP1-α and MIP-1β), against CD19-expressing cell lines and, when available, primary CD19+ tumor cells collected and stored from recipients prior to treatment.

Our preliminary data predict that we will be able to generate sufficient numbers of cells to meet the dose requirements of the proposed study,[62] and that the CB-derived NK cells will have specificity against B-lymphoid malignancies (including ALL, CLL, NHL), without introducing unacceptable toxicity (**Fig. 4-8**).

Even so, we are aware of several pitfalls that could compromise this projected outcome. **Cytokine Release Syndrome**. There have been recent reports of potentially life-threatening cytokine storm-related side-effects after adoptive therapy with CAR-transduced T cells, while prolonged B cell aplasia is a well-documented on-target toxicity of CAR19-transduced T cells.[63;64] We will use the criteria for diagnosis and management defined in Appendices A. and B. to manage such patients. Moreover, to counteract these potential complications, we have incorporated a suicide gene based on the inducible caspase-9 gene into the CAR19 vector.[54] As shown in **Fig 8**, the addition of a small molecule dimerizer, AP1903, induces rapid apoptosis of transgenic cells, such that in the case of prolonged B lymphopenia, the dimerizer could be introduced to induce apoptosis of CAR19-transduced CB-NK cells, allowing normal recovery of B cells. This strategy would also be useful if the transduced NK cells are found to induce GVHD.

3.0 Patient Eligibility

Inclusion criteria:

1. Patients with history of CD19 positive B-lymphoid malignancies, defined as acute lymphoblastic leukemia (ALL), diffuse large B-cell Lymphoma (DLBCL), chronic lymphocytic leukemia (CLL) small lymphocytic lymphoma (SLL), primary mediastinal large B-cell lymphoma, Richter's transformation of CLL or SLL, follicular lymphoma, marginal zone lymphoma and high grade transformation of follicular or marginal zone lymphoma who have received at least 2 lines of standard chemoimmunotherapy or targeted therapy and have persistent disease.
2. Patients with CD19 positive B-lymphoid malignancies as defined above with relapsed disease following standard therapy or a stem cell transplant.
3. Patients at least 3 weeks from last cytotoxic chemotherapy at the time of starting lymphodepleting chemotherapy. Patients may continue tyrosine kinase inhibitors or other targeted therapies until at least three days prior to administration of lymphodepleting chemotherapy.
4. Karnofsky/Lansky Performance Scale > 70.
5. Adequate organ function:
    a. Renal: Serum creatinine </= 1.5 mg/dL or estimated Glomerular Filtration Rate (eGFR using the CKI-EPI equation) >/= 60 ml/min/1.73 m^2.
    b. Hepatic: ALT/AST </= 2.5 x ULN or </= 5 x ULN if documented liver metastases, Total bilirubin </= 1.5 mg/dL, except in subjects with Gilbert's Syndrome in whom total bilirubin must be </= 3.0 mg/dL. No history of liver cirrhosis. No ascites.
    c. Cardiac: Cardiac ejection fraction >/= 50%, no clinically significant pericardial effusion as determined by an ECHO or MUGA, and no clinically significant ECG findings. Clinical significance attributed per PI discretion.
    d. Pulmonary: No clinically significant, per PI discretion, pleural effusion, baseline oxygen saturation > 92% on room air.
6. Able to provide written informed consent.

7. 7-80 years of age.
8. All participants who are able to have children must practice effective birth control while on study and up to 3 months post completion of study therapy. Acceptable forms of birth control for female patients include: hormonal birth control, intrauterine device, diaphragm with spermicide, condom with spermicide, or abstinence, for the length of the study. If the participant is a female and becomes pregnant or suspects pregnancy, she must immediately notify her doctor. If the participant becomes pregnant during this study, she will be taken off this study. Men who are able to have children must use effective birth control while on the study. If the male participant fathers a child or suspects that he has fathered a child while on the study, he must immediately notify his doctor.
9. Signed consent to long-term follow-up protocol PA17-0483.

Exclusion Criteria:

1. Positive beta HCG in female of child-bearing potential defined as not postmenopausal for 24 months or no previous surgical sterilization or lactating females.
2. Known positive serology for HIV.
3. Presence of clinically significant Grade 3 or greater toxicity from the previous treatment, as determined by PI.
4. Presence of fungal, bacterial, viral, or other infection requiring IV antimicrobials for management. Note: Simple UTI and uncomplicated bacterial pharyngitis are permitted if responding to active treatment.
5. Presence of active neurological disorder(s).
6. Concomitant use of other investigational agents.
7. Previously received any anti-CD19 therapy (for example, CAR treatment or blinatumomab).

4.0 Treatment Plan

Lymphodepleting Chemotherapy (inpatient):

On or before D-15	Begin NK cell production
D -6	Admit / IV Hydration
D -5	Fludarabine 30 mg/m^2 IV / Cyclophosphamide 300 mg/m^2 IV / Mesna 300 mg/m^2 IV
D -4	Fludarabine 30 mg/m^2 IV / Cyclophosphamide 300 mg/m^2 IV / Mesna 300 mg/m^2 IV
D -3	Fludarabine 30 mg/m^2 IV / Cyclophosphamide 300 mg/m^2 IV / Mesna 300 mg/m2 IV
D -2	Rest
D -1	Rest
D0	Infusion of iC9/CAR.19/IL15-transduced CB-NK cells

Between D7 & 14	Infusion of iC9/CAR.19/IL15-transduced CB-NK cells*

Lymphodepleting Chemotherapy (outpatient):

On or before D-15	Begin NK cell production
D -5	Fludarabine 30 mg/m^2 IV / Cyclophosphamide 300 mg/m IV / Mesna 300 mg/m^2 IV
D -4	Fludarabine 30 mg/m^2 IV / Cyclophosphamide 300 mg/m^2 IV / Mesna 300 mg/m^2 IV
D -3	Fludarabine 30 mg/m^2 IV / Cyclophosphamide 300 mg/m^2 IV / Mesna 300 mg/m^2 IV
D-2	Rest
D-1	Rest
D0	Infusion of iC9/CAR.19/IL15-transduced CB-NK cells
Between D7 & 14	Infusion of iC9/CAR.19/IL15-transduced CB-NK cells*

* If no DLT is observed following the initial NK cell infusion, patients with bulky disease or rapidly progressive disease at study entry can receive at the investigators discretion a 2nd NK cell infusion between days 7 and 14, using the same NK cell dose given initially, .

Cell dose.
Adult patients (i.e. 18 years or older) who weigh 40 kilograms or more will receive a flat dose of 800 x10^6 ± 200x10^6 iC9/CAR.19/IL15-transduced CB-NK cells. Adult patients who weigh less than 40 kilograms and pediatric patients (younger than 18 years) will receive a dose of 10^7 iC9/CAR.19/IL15-transduced CB-NK cells per kilogram of weight.

If the patient has relapsed or has persistent disease after a protocol assessment, an additional CAR NK infusions may be given. If there are left over cells from their first production, they may be used or a new cord unit may be selected for CAR NK generation. Prescreening testing will not need to be repeated if within 45 days of the previous tests, or at physician discretion. The lymphodepleting chemotherapy should be repeated if the additional infusion is given beyond 21 days of the date of completion of the last lymphodepleting chemotherapy. Patients with bulky or rapidly progressive disease can receive 2 consecutive infusions as described above

Cyclophosphamide is dosed per adjusted body weight for patients weighing > 20%

above their ideal body weight. For patients less than or equal to 20% above their ideal body weight, the actual body weight is used.

On D 0 the NK cell infusion will be administered intravenously. Premedicate with Benadryl 25 mg po or IV and Tylenol 650 mg po. The use of steroids is contraindicated unless required for physiologic replacement.

Subjects may be either inpatient or outpatient for the CAR NK infusion, depending upon bed availability and/or patient's clinical situation. Vital signs (temperature, heart rate, blood pressure, and respiratory rate) will be obtained on all patients per BMT standard of care for cellular therapy which includes at the start of the CAR NK infusion and approximately every 15 minutes x 4 followed by approximately every 30 minutes x 2 or until 1 hour after completion of the CAR NK infusion. Then approximately every hour as indicated by patient's condition.

NK cells will be obtained by the following method:
Frozen cord blood units will be thawed and mononuclear cells will be isolated by Ficoll density gradient centrifugation. NK cells will be CAR transduced and generated for 14 to 22 days in liquid cultures using APC feeder cells as described in detail in the Chemistry, Manufacturing and Controls (CMC).

NK Product Release Criteria
The following minimum criteria will be required for release of the expanded NK cells for reinfusion:

Stat Gram Stain: "No Organisms Seen". CAR+ NK cells: > 15%
CD3+ number: < 2 e5 CD3+ cells/kg. CD32+ cell number (aAPV): < 5%
NK cells (CD16+/56+): >80%
Visual Inspection: "No Evidence of Contamination" (turbidity; change in media color).
Endotoxin Assay: < 5EU/Kg.
Viability: $\geq$ 70%.

Other parameters which will be monitored include sterility culture for bacteria and fungi. If more than 2 x 10e5 CD3+ cells/kg are present, a second cycle of CD3 depletion may be performed. The cell dose for infusion may be reduced so that the infused CD3+ cells are $\leq 2 \times 10^5$/ kg.

If adequate CAR+ NK cell dose is not generated, then all available cells will be infused. If this occurs for patients in the MTD finding stage of this study, they will not be counted in any cohort. If more than the required NK dose is generated, the additional NK cells may be cryopreserved for future infusions or may be used for research.

If CAR NK cells cannot be released due to microbial contamination, another cord blood unit will be selected and production will start over. If the patient has already completed lymphodepleting chemotherapy, they may require a second dose of lymphodepleting chemotherapy prior to the CAR NK infusion.

Administration of the Dimerizer AP1903 for Cytokine Release Syndrome (CRS), Neurotoxicity, or GVHD.

The MD Anderson CARTOX committee has developed the detailed algorithms for the management of CRS or neurotoxicity summarized in Appendix A. We will follow the algorithm rigorously with the administration of Tocilizumab 8 mg/kg IV q 6h as needed for up to 3 doses / 24h for Grade 2 CRS or Grade 2 Neurotoxicity not responding to standard supportive measures. For Grade 3 CRS and Grade 3 Neurotoxicity as defined in the CARTOX algorithm, in addition to the Tocilizumab, a single dose of AP1903 will be administered (0.4 mg/kg as an intravenous infusion over approximately 2 hours). The AP1903 dose is based on published Pk data which show plasma concentrations of 10-1275 ng/mL over the 0.01 mg/kg to 1.0 mg/kg dose range with plasma levels falling to 18% and 7% of maximum at 0.5 and 2hrs post dose.[65] The dimerizer can also be used for the treatment of grades I-IV GVHD. Responses in patients with GVHD who had received Capsase-9+ T cells and then the AP1903, responses have occurred within the first 24-48 hours.

Patients who do not experience downgrading or CRS or neurotoxicity to Grade 2 or less within 12 hours may receive a second dose of AP1903 but will also receive high dose steroids as outlined in the CARTOX Algorithm in Appendix A. and Appendix B.

Birth Control Specifications

All participants who are able to have children must practice effective birth control while on study and up to 3 months post completion of therapy. Acceptable forms of birth control for female patients include: hormonal birth control, intrauterine device, diaphragm with spermicide, condom with spermicide, or abstinence, for the length of the study. If the participant is a female and becomes pregnant or suspects pregnancy, she must immediately notify her doctor. If the participant becomes pregnant during this study, she will be taken off this study. Men who are able to have children must use effective birth control while on the study. If the male participant fathers a child or suspects that he has fathered a child while on the study, he must immediately notify his doctor.

Additional Therapies

If the patient has persistent disease after a protocol assessment, standard of care therapies may be given at discretion of the physician and PI. The patient will remain on study to monitor for persistence of CAR NK cells and may be considered for an additional infusion.

5.0 Evaluation During Study

Evaluation During Study

Every effort will be made to adhere to the schedule of events and all protocol requirements. Variations in schedule of events and other protocol requirements that do not affect the rights and safety of the patient will not be considered as deviations. Such variations may include laboratory assessments completed outside of schedule and occasional missed required research samples. Missed samples for correlative studies

will not constitute protocol deviations.

5.1 Evaluation: Any time before or during study: HLA typing (high resolution A,B, DR).

5.2 The following evaluations will be obtained within 30 days of study enrollment:

5.2.1 History and physical examination
5.2.2 CBC w/diff and platelets, total bilirubin, SGPT, alkaline phosphatase, LDH, albumin, total protein, BUN, creatinine, glucose, electrolytes, PT/PTT, type and screen, immunoglobulin levels (IGG, IGM, IGA), and cytokine panel 3 (IL6, IFN gamma, TNF alpha).
5.2.3 Serology for HIV.
5.2.4 ECHO or MUGA.
5.2.5 Pulmonary function tests, if clinically indicated.
5.2.6 Chest x-ray
5.2.7 Urinalysis.
5.2.8 CT brain.
5.2.9 PET/CT scan as clinically indicated.
5.2.10 Bone marrow aspiration as clinically indicated.
5.2.11 EKG

5.3 The following blood will be drawn after the consent is signed but before the lymphodepleting chemotherapy is initiated:

5.3.1 Research Labs, consisting of 60 mLs of blood (5 x green top and 1 purple top) will be drawn and sent to Dr. Rezvani's laboratory. The studies will be performed free of charge to the patient.

5.4 Evaluations within 7 days of starting lymphodepleting chemotherapy:

5.4.1 History and physical examination including weight and vital signs.
5.4.2 Laboratory examinations: CBC w/diff and platelets, total bilirubin, SGPT, alkaline phosphatase, LDH, albumin, total protein, BUN, creatinine, glucose, electrolytes, and cytokine analysis. Serum pregnancy test if female participant of childbearing potential.

5.5 The following evaluations will be obtained on approximately: day 0, day 3, day 7, day 14, and day 21, week 4, week 8, week 12, week 16, month 6, month 9, and 1 year after CAR-NK infusion:

5.5.1 Physical examination including weight and vital signs at Day 7 only.
5.5.2 CBC w/diff and platelets, chemistry panel, and cytokine analysis.
5.5.3 Cytokine panel 3 (IL6, IFN gamma, TNF alpha) at all time points, except only as clinically indicated at approximately month 6, month 9, and month 12.
5.5.4 HLA antibodies at approximately 4 weeks and 12 weeks only.
5.5.5 Research Labs: CAR NK detection, phenotype and function to be performed in Dr. Katayoun Rezvani's Laboratory as above.

5.6 The following evaluations will be obtained on approximately week 4, week 8, week 12, week 16, and month 6, month 9, and month 12 after CAR-NK infusion:

5.6.1 PET/CT scan as clinically indicated.

5.7 The following evaluations will be obtained on approximately day 7, week 4, week 8, week 12, week 16, month 6, month 9, and 1 year after CAR-NK infusion:

5.7.1 Bone marrow aspiration and/or biopsy as clinically indicated.
5.7.2 Research Labs: 5 to 10 mL bone marrow aspirate will be sent to Dr. Rezvani's Laboratory, as clinically indicated.

5.8 Additional lab tests

Additional lab draws may be needed to monitor the car NK cells and patient disease status. The patient may be asked to sign a separate consent for protocol LAB00-099 for these additional samples. Analysis will be performed in Dr. Katy Rezvani's Lab free of charge for the patient. Analysis results from these samples performed under protocol LAB00-099 will be used in protocol 2016-0641.

5.9 Lymph node biopsy

If the patient has a diagnostic lymph node biopsy, a portion of the specimen may be sent to Dr. Katy Rezvani's Lab for analysis, if available.

Table 1- Table of Evaluations

	Pre	Day 0	3 days	7 days	14 days	21 days	4 weeks	Day 40	8 weeks	12 weeks	16 weeks	6, 9, 12 months
Physical Exam	x[1]			x[1]								
CBC, chem panel	x[1]		x	x	x	x	x		x	x	x	x[2]
HIV serology	x											
Cytokine Panel[3]		x[4]		x	x	x	x		x	x	x	x[2]
HLA antibodies							x			x		
Research labs: CAR NK detection, phenotype and function	x	x[6]	x	x	x	x	x		x	x	x	x[2]
Cytokine Analysis[3]	x		x	x	x	x	x		x	x	x	x[2]
Chimerism[3]			x	x	x	x	x		x	x	x	x[2]
RCR blood test[5]							x			x		x[2]
Pregnancy Test[2]	x[1]											
Urinalysis	x											
EKG	x											
ECHO or MUGA	x											
Pulmonary Function Test	x[2]											
Brain CT	x											
PET/CT	x						x[2]		x[2]	x[2]	x[2]	x[2]
Bone marrow analysis	x[2]			x[2]			x[2]		x[2]	x[2]	x[2]	x[2]
AE assessment		< ========== >										
Bone marrow research labs to assess NK CAR homing	x			x			x[2]		x[2]	x[2]	x[2]	x[2]

Time frame windows: Protocol assessments will be done approximately on days listed above. For patients who receive a second CAR NK infusion, the post testing timepoints will be based from date of second infusion.

[1] Physical Exam, CBC, chem panel: Within 30 days and 7 days of starting lymphodepleting chemotherapy. Pregnancy test: Within 7 days of starting lymphodepleting chemotherapy. Physical at approximately Day 7 only.

[2] As clinically indicated.

[3] Drawn as part of the research lab z code. Samples will be batched and run approximately every 3 months for results.

[4] Cytokine Panel 3: Day 0 prior to CAR NK infusion.

[5] Replication-competent retrovirus (RCR): About 1, 3, 6 and 12 months post NK cell infusion, and then per long term follow up study PA17-0483.

[6] On day 0 a research sample will be drawn before infusion, may be with morning labs, and then approximately 1-3 hours after completion.

6.0 Background Drug Information

The medications used in this protocol will be obtained through a commercially available supply.

Cyclophosphamide:

Therapeutic Classification: Antineoplastic alkylating agent

Pharmaceutical Data: Lyophilized cyclophosphamide for injection contains 75 mg of mannitol per 100 mg of cyclophosphamide (anhydrous) and is supplied in vials for single dose use.

Stability and Storage: Storage at or below 77°F (25°C) is recommended; this product will withstand brief exposure to temperatures up to 86°F(30°C) but should be protected from temperatures above 86°F. Reconstituted lyophilized cyclophosphamide for injection is chemically and physically stable for 24 hours at room temperature or for 6 days in the refrigerator; it does not contain any antimicrobial preservative and thus care must be taken to assure the sterility of prepared solutions.

Mechanism of Action: It interferes with DNA replication and transcription of RNA and ultimately results in the disruption of nucleic acid function.

Side effects: Dose limiting toxicity is hematologic. Nausea and vomiting are common. Anorexia, less frequently abdominal discomfort or pain and diarrhea may occur. There are isolated reports of hemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy. Azotemia has been reported. Alopecia and hypersensitivity reactions can occur when high doses are utilized. Hemorrhagic cystitis may develop in patients treated with cyclophosphamide. Rarely this condition can be severe and even fatal. Acute cardiotoxicity has been reported with doses as low as 2.4 g/m Interstitial pulmonary fibrosis has been reported in patients receiving high doses of cyclophosphamide over a prolonged period.

Fludarabine:

Therapeutic Classification: Antineoplastic agent; Antimetabolite (Purine Analog)

Pharmaceutical Data: Each vial contains 50 mg lyophilized drug, to be reconstituted with 2 ml sterile water to a solution that is 25 mg/ml for IV administration.

Solution Preparation: Mix each vial with 2 ml sterile pyrogen-free water to a clear solution, which is 25 mg/ml for IV administration only. Reconstituted solution should be used within 8 hours.

Adverse Effects:
Observed in more than 10%: Cardiovascular: Edema (8% to 19%); Central nervous system: Fever (11% to 69%), fatigue (10% to 38%), pain (5% to 22%), chills (11% to 19%); Dermatologic: Rash (4% to 15%); Gastrointestinal: Nausea/vomiting (1% to 36%), anorexia (34%), diarrhea (5% to 15%), gastrointestinal bleeding (3% to 13%); Genitourinary: Urinary tract infection (2% to 15%); Hematologic: Myelosuppression

(nadir: 10-14 days; recovery: 5-7 weeks; dose-limiting toxicity), anemia (14% to 60%), neutropenia (grade 4: 37% to 59%; nadir: ~13 days), thrombocytopenia (17% to 55%; nadir: ~16 days); Neuromuscular & skeletal: Weakness (9% to 65%), myalgia (4% to 16%), paresthesia (4% to 12%); Ocular: Visual disturbance (3% to 15%); Respiratory: Cough (£44%), pneumonia (3% to 22%), dyspnea (1% to 22%), upper respiratory infection (2% to 16%), rhinitis (£11%); Miscellaneous: Infection (12% to 44%), diaphoresis (£14%)

Observed in 1% to 10%: Cardiovascular: Peripheral edema (less than 7%), angina (less than 6%), chest pain (less than 5%), CHF (3%), arrhythmia (3%), cerebrovascular accident (3%), MI (3%), supraventricular tachycardia (3%), deep vein thrombosis (1% to 3%), phlebitis (1% to 3%), aneurysm (less than 1%), transient ischemic attack (1%); Central nervous system: Headache (9%), malaise (6% to 8%), sleep disorder (1% to 3%), cerebellar syndrome (less than 1%), depression (1%), mentation impaired (1%); Dermatologic: Alopecia (3%), pruritus (1% to 3%); Endocrine & metabolic: Hyperglycemia (1% to 6%), LDH increased (less than 6%), dehydration (1%); Gastrointestinal: Abdominal pain (10%), stomatitis (9%), weight loss (6%), esophagitis (3%), constipation (1% to 3%), mucositis (2%), dysphagia (1%); Genitourinary: Dysuria (3% to 4%), hesitancy (3%); Hematologic: Hemorrhage (1%), myelodysplastic lyndrome/acute myeloid leukemia (usually associated with prior or concurrent treatment with other anticancer agents); Hepatic: Cholelithiasis (3%), liver function tests abnormal (1% to 3%), liver failure (1%); Neuromuscular & skeletal: Back pain(9%), osteoporosis (2%), arthralgia (1%); Otic: Hearing loss (2% to 6%); Renal: Hematuria (2% to 3%), renal failure (1%), renal function test abnormal (1%), proteinuria (1%); Respiratory: Bronchitis (9%), pharyngitis (9%), allergic pneumonitis (6%), hemoptysis (1% to 6%), sinusitis (5%), epistaxis (1%), hypoxia (1%); Miscellaneous: Flu-like syndrome (5% to 8%), herpes simplex infection (8%), anaphylaxis (1%), tumor lysis syndrome (1%)

Observed in <1%, postmarketing, and/or case reports: Acute respiratory distress syndrome, agitation, blindness, blurred vision, bone marrow fibrosis, coma, confusion, diplopia, eosinophilia, Epstein-Barr virus (EBV) associated lymphoproliferation, EBV reactivation, erythema multiforme, Evans syndrome, flank pain, hemolytic anemia (autoimmune), hemophilia (acquired), hemorrhagic cystitis, herpes zoster reactivation, hyperkalemia, hyperphosphatemia, hyperuricemia, hypocalcemia, interstitial pneumonitis, metabolic acidosis, opportunistic infection, optic neuritis, optic neuropathy, pancreatic enzymes abnormal, pancytopenia, pemphigus, pericardial effusion, peripheral neuropathy, photophobia (primarily with high doses), progressive multifocal leukoencephalopathy (PML), pulmonary fibrosis, pulmonary hemorrhage, pulmonary infiltrate, respiratory distress, respiratory failure, Richter's syndrome, seizure, skin cancer (new onset or exacerbation), Stevens-Johnson syndrome, thrombocytopenia (autoimmune), thrombocytopenic purpura (autoimmune), toxic epidermal necrolysis, trilineage bone marrow aplasia, trilineage bone marrow hypoplasia, urate crystalluria, wrist drop

Also observed: Neurologic syndrome characterized by cortical blindness, coma, and paralysis [36% at doses >96 mg/m^2 for 5-7 days; <0.2% at doses <125 mg/m^2 /cycle (onset of neurologic symptoms may be delayed for 3-4 weeks)]

Mechanism of Action: Fludarabine inhibits DNA synthesis by inhibition of DNA polymerase and ribonucleotide reductase; also inhibits DNA primase and DNA ligase I.

Human Safety and Pharmacology.

Distribution: Vd: 38-96 L/m2; widely with extensive tissue binding

Protein binding: 2-fluoro-ara-A: ~19% to 29%
Metabolism: I.V.: Fludarabine phosphate is rapidly dephosphorylated in the plasma to 2-fluoro-ara-A (active metabolite), which subsequently enters tumor cells and is phosphorylated by deoxycytidine kinase to the active triphosphate derivative (2-fluoro-ara-ATP)
Bioavailability: Oral: 2-fluoro-ara-A: 50% to 65% **Half-life elimination**: 2-fluoro-ara-A: ~20 hours **Time to peak, plasma**: Oral: 1-2 hours
Excretion: Urine (60%, 23% as 2-fluoro-ara-A) within 24 hours

Dose adjustment in renal and hepatic impairment: It appears that no adjustment is needed in hepatic impairment. Renal impairment dosing is **NOT** specific to stem cell transplant patients. In patients not receiving a stem cell transplant, doses are typically reduced by 20% for CrCl of 30-70 ml/min and not used for CrCl < 30 ml/min.

Monitoring Parameters: CBC with differential, platelet count, AST, ALT, serum creatinine, serum albumin, uric acid; monitor for signs of infection and neurotoxicity

Mesna (sodium -2-mercapto ethane sulphonate)

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazophosphorines (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazophosphorines. At the doses used for uroprotection, mesna is virtually non-toxic. However, adverse effects which may be attributable to mesna include nausea and vomiting, diarrhea, abdominal pain, altered taste, rash, urticaria, headache, joint or limb pain, hypotension and fatigue.

7.0 Criteria for Removal from the Study

1. Patient withdrawal of the informed consent/authorization, or refusal to continue on study.
2. An increasing or unexpected pattern of toxicity is observed deemed unacceptable by the PI, co-PI, or designee.
3. Inability to infuse CAR-NK cell product due to product manufacturing issues.
4. Study completion at one year.
5. Death.
6. Not able to receive CAR NK infusion(s).
7. Non-compliance with study procedures and monitoring.
8. Lost to follow-up.
9. Disease progression,
10. If for any reason, the PI feels it in the best interest of the patient to come off study.

8.0 Statistical Considerations

1. Preliminaries This is a single-arm phase II trial of cord blood derived CAR CD-19 natural killer (NK) cells, administered at a dose of 800x10e6 cells to patients with any hematologic malignancy who have either relapsed or have been declared refractory after initial chemotherapy. The primary scientific goal of the trial is to estimate the probabilities of treatment efficacy and toxicity.

2. Outcomes.

Toxicity is defined as grade 3 or 4 GVHD within 40 days of NK cell infusion or cytokine release syndrome (CRS) within 2 weeks of NK cell infusion requiring transfer to intensive care.

Efficacy is defined as the patient being alive and in at least partial remission at day 30 post NK cell infusion.

Secondary outcomes. Secondary outcomes will include progression-free survival (PFS) time, overall survival (OS) time, and response at day 100.

3. Maximum Sample Size and Cohort Size. A maximum of 120 patients will be treated in up to 8 cohorts of size 15 patients each. This sample size ensures that, if for example 36/120 (30%) of the patients have the Efficacy outcome, then starting with a beta(.30, .70) prior, a posterior 95% credible interval for Pr(Efficacy) would be .22 - .38 . Similarly, if 48/120 patients experience toxicity, then starting with a beta(.40, .60) prior, a posterior 95% credible interval for Pr(Toxicity) would be .31 - .49.

4. Safety and Futility Monitoring. The method of Thall et al[69] will be used to construct two early stopping bounds, one for inadequate efficacy and one for excessive toxicity, that will be applied simultaneously. For the purpose of monitoring Efficacy and Toxicity, a maximum upper limit on Pr(Toxicity) will be .40, and a minimum lower limit on Pr(Efficacy) will be .30. Note that, because Efficacy includes 30-day survival as a subevent, monitoring the probability of Efficacy includes monitoring of early death.

Assuming that Efficacy and Toxicity and independent events, the four composite elementary events will be denoted by A1 = [Efficacy and Toxicity], A2 = [Efficacy and No Toxicity], A3 = [No Efficacy and Toxicity], and A4 = [No Efficacy and No Toxicity]. Thus, Efficacy = A1 U A2 and Toxicity = A1 U A3. Denoting the respective probabilities of these 4 elementary events with the experimental treatment E = cord blood derived CAR CD-19 natural killer (NK) cells, by q = (q1, q2, q3, q4), so that q1 + q2 + q3 +q4 = 1, As a standard, S, for comparison, the historical treatment distribution for use as a comparator, with probabilities p = (p1, p2, p3, p4). With E, the probability of Efficacy be denoted by qE = q1 + q2, and the probability of Toxicity will be denoted by qT = q1 + q3. With S, the probability of Efficacy be denoted by pE = p1 + p2, and the probability of Toxicity will be denoted by pT = p1 + p3. It will be assumed, conservatively, that q follows a Dirichlet prior distribution with parameters (.12, .18, .28, .42), implying that qE follows a beta prior with parameters (.30, .70). It will be assumed that p follows a Dirichlet distribution with parameters (120, 180, 280, 420).

The trial will be stopped early for lack of Efficacy if Pr(pE < qE | data) < .01

The trial will be stopped early for unacceptable Toxicity if Pr(pT < qT | data) > .99

These posterior probability criteria imply that the trial will be stopped early if either

[number of patients with efficacy] / [number of patients evaluated] is less than or equal to

0/15, 3/30, 6/45, 9/60, 13/75, 16/90, or 20/105,

or

[number of patients with toxicity] / [number of patients evaluated] is greater than or equal to

11/15, 19/30, 27/45, 34/60, 41/75, 48/90, or 55/105.

5. Operating Characteristics

The design's operating characteristics are summarized in Table 1. **The two monitoring rules will be applied after successive cohorts of 15** without halting the enrolment while the data is being analyzed. The simulations were carried out using multc99.

Table 1. Design operating characteristics, based on Nmax = 120, with the two monitoring rules applied after successive cohorts of 15, at interim sample sizes of 15, 30, 45, 60, 75, 90 and 105.

Scenario	p1	p2	p3	p4	Pr(Eff)	Pr(Tox)	Pr(Stop Early)	Sample Size 25th, 50th, 75th percentiles
1	.12	.18	.28	.42	.30	.40	0.06	120 120 120
2	.05	.05	.35	.55	.10	.40	1.00	30 30 45
3	.12	.18	.48	.22	.30	.60	0.96	30 45 60
4	.05	.05	.55	.35	.10	.60	1.00	15 30 30

In scenario 1, both Pr(Eff) and Pr(Tox) are acceptable.

In scenario 2, Pr(Eff) = .10 is too low.

In scenario 3, Pr(Tox) = .60 is too high.

In scenario 4, both Pr(Eff) and Pr(Tox) are unacceptable.

6. Data analyses

Unadjusted distributions of the time-to-event outcomes OS and PFS will be estimated using the method of Kaplan and Meier.[70] Their relationship to prognostic covariates and OS or pFS will be evaluated by Bayesian piecewise exponential survival regression.[71]

7. Criteria for Response

For the purpose of the Efftox assessment, a response is defined as a partial or complete response.

- Acute lymphoblastic leukemia: Complete response will be defined as bone marrow with < 5% blasts, the absence of circulating blasts, and no extramedullary sites of disease (as assessed by means of computed tomography or positron-emission tomography), regardless of cell-count recovery.
- CLL: Response will be defined based on the NCI-WG/IWCLL 2008 criteria, *Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute–Working Group 1996 guidelines*.[72]

  Lymphomas: Response will be defined based on the *Lugano criteria- Recommendations for Initial Evaluation, Staging, and Response Assessment of Hodgkin and Non-Hodgkin Lymphoma: The Lugano Classification*.[73]

The Investigator is responsible for completing toxicity summary reports and submitting them to the IND office Medical Monitor for review. These should be submitted as follows:

After the first 15 evaluable patients, complete 40 days of study treatment, and every 15 evaluable patients thereafter.

9.0 Adverse Events and Reporting Requirements

An Adverse Event is defined as any untoward medical occurrence in a patient regardless of its causal relationship to study treatment. An AE can be any unfavorable and unintended sign (including any clinically significant abnormal laboratory test result), symptom, or disease temporally associated with the use of the study treatment, whether or not it is considered to be study drug(s) related. Included in this definition are any newly occurring events and any previous condition that has increased in severity or frequency since the administration of study therapy.

Attribution - the determination of whether an adverse event is related to a medical treatment or procedure.

- **Definite** - the adverse event is clearly related to the investigational agent(s).
- **Probable** - the adverse event is likely related to the investigational agent(s).
- **Possible** - the adverse event may be related to the investigational agent(s).
- **Unlikely** - The adverse event is doubtfully related to the investigational agent(s).
- **Unrelated** - The adverse event is clearly NOT related to the investigational agent(s).

For the purpose of this study, the investigational component of the treatment plan is the infusion of ex vivo-expanded, genetically modified, iC9/CAR.19/IL15-transduced NK cells. Therefore, serious and unexpected adverse events occurring from the infusion of the iC9/CAR.19/IL15-transduced NK cells and up to 30 days of the last treatment

intervention, as defined below, will be reported according to MDACC policy and procedures below and the (OBA) Serious Adverse Event Reporting Form for Human Gene Transfer Clinical Studies at http://www4.od.nih.gov/oba/rac/documents1.htm.

The MDACC Internal Adverse Event Reporting Form will be used for reporting to the IRB and the Investigational New Drug (IND) Office. Serious adverse events must be followed until clinical recovery and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event. Additionally, any serious adverse events occurring after the 30 day time period that are related to the study treatment must be reported to the IRB and the IND Office. This includes the development of a secondary malignancy.

Hospitalizations for treatment of disease, or related to complications from treatment administered prior to CAR infusion will not be captured.

Collection of adverse events (AEs) will begin at the time of the iC9/CAR.19/IL15-transduced NK cell infusion and will reflect the onset and resolution date and maximum grade. BMTWeb/CORe will be used as the electronic case report form for this protocol, and all protocol specific data will be entered into BMTWeb/CORe. All protocol specific data defined as abnormal physical exam and/or laboratories and AEs related to iC9/CAR.19/IL15-transduced NK cell infusion will be entered into BMTWeb/CORe. Intermittent events should be labeled as such and followed until resolution. If a patient is taken off study while an event is still ongoing, this will be followed until resolution unless another therapy is initiated. Additionally, patients may be on multiple concurrent medications that are not necessarily related to the iC9/CAR.19/IL15-transduced NK cell infusion. These medications are considered standard of care and have no scientific contributions to the protocol, therefore concurrent medications will not be captured.

MD Anderson's Clinical Research Serious Adverse Events Reporting (eSAE) system will be used to collect and report all serious adverse event information.

Assessment of adverse events
The Investigator or physician designee is responsible for verifying and providing source documentation for all adverse events and assigning the attribution for each event for all subjects enrolled on the trial.

Adverse events related to the iC9/CAR.19/IL15-transduced NK cell infusion and protocol specific data will be entered into PDMS/CORe. PDMS/CORe will be used as the electronic case report form for this protocol.

Severity of the adverse events (AEs)
All grades of AEs related to iC9/CAR.19/IL15-transduced NK cell infusion will be collected. All AEs will be recorded on the AE log. Adverse events unrelated to the iC9/CAR.19/IL15-transduced NK cell infusion will not be collected.

Events not included in the CTCAE chart will be scored as follows:

General grading:
Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment

required beyond prophylaxis.
Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment. Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment.
Grade 4: Life Threatening: discomfort that represents immediate risk of death

Causality Assessment
The investigational component of the treatment plan of this study is the infusion of ex-vivo expanded iC9/CAR.19/IL15-transduced NK cells after lymphodepleting chemotherapy.

Therefore, events known to be caused by the iC9/CAR.19/IL15-transduced NK cell infusion and its direct consequences will be assessed as definitely related when assessing the causality.
When the relationship of the adverse event cannot be rule out between the iC9/CAR.19/IL15-transduced NK cell infusion and the chemotherapy, the event will be scored as probably or possible related.

Events known to be related to drugs used as chemotherapy as well as to drugs used as supportive treatment will be scored as unrelated to the iC9/CAR.19/IL15-transduced NK cell infusion.

The principal investigator will be the final arbiter in determining the causality assessment.

AEs related to the iC9/CAR.19/IL15-transduced NK cell infusion:
1. These expected events will be monitored at approximately 30 minutes and 60 minutes post infusion:
- Fever,
- Chills,
- Decrease in blood pressure,
- Rash,
- Shortness of breath.

2. Dose limiting, (also considered unexpected):
- Grade 4 NK infusion related toxicity,
- Grades 3-5 allergic reactions related to study cell infusion,
- Grade 3-5 organ toxicity (cardiac, dermatologic, gastrointestinal, hepatic, pulmonary, renal/genitourinary, or neurologic) not pre-existing or due to the underlying malignancy or due to lymphodepleting chemotherapy
- Grades 3-4 acute GVHD occurring within 8 weeks.
- Treatment-related death within 8 weeks of the study cell infusion.

Adverse Events Considered Serious:
- Grade 4 NK infusion related toxicity.
- GvHD.
- Prolonged hospitalization due to infections and/or organ failure requiring extensive supportive care (i.e. dialysis, mechanical ventilation).
- Any expected or unexpected event resulting in an irreversible condition and/ or leading to death.

Abnormal Laboratory Findings

For the purpose of this study, abnormal laboratory findings considered associated to the original disease as well as isolated changes in laboratory parameters such as electrolyte magnesium and metabolic imbalances, uric acid changes, elevations of GPT, GOT, LDH, alkaline phosphatase, and CBC would not be considered adverse events and will not be collected in the database or toxicity logs.

Concomitant Medications

Patients treated on this protocol will require supportive care treatment (concomitant medications). These medications are considered standard of care and have no scientific contribution to the protocol; therefore no data will be captured on various medications needed or their side effects.

Serious Adverse Event Reporting (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or the sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience – any adverse experience that places the patient, in the view of the initial reporter, at immediate risk of death from the adverse experience as it occurred. It does not include an adverse experience that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse (21 CFR 312.32).

- **Important medical events as defined above, may also be considered serious adverse events. Any important medical event can and should be reported as an SAE if deemed appropriate by the Principal Investigator or the IND Sponsor, IND Office.**

- All events occurring during the conduct of a protocol and meeting the definition of a SAE must be reported to the IRB in accordance with the timeframes and procedures outlined in “The University of Texas M. D. Anderson Cancer Center Institutional Review Board Policy for Investigators on Reporting Unanticipated Adverse Events for Drugs and Devices”.

Unless stated otherwise in the protocol, all SAEs, expected or unexpected, must be reported to the IND Office, regardless of attribution (within 5 working days of knowledge of the event).

- **All life-threatening or fatal events,** that are unexpected, and related to the study drug, must have a written report submitted within **24 hours** (next working day) of knowledge of the event to the Safety Project Manager in the IND Office.

- **Unless otherwise noted, the electronic SAE application (eSAE) will be utilized for safety reporting to the IND Office and MDACC IRB.**

- **Serious adverse events will be captured from the time of the first protocol-specific intervention, until 30 days after the last dose of drug, unless the participant withdraws consent. Serious adverse events must be followed until clinical recovery is complete and laboratory tests have returned to baseline, progression of the event has stabilized, or there has been acceptable resolution of the event.**

- **Additionally, any serious adverse events that occur after the 30 day time period that are related to the study treatment must be reported to the IND Office. This may include the development of a secondary malignancy.**

- **The gene therapy reporting addendum ("Additional Reporting Form for Serious Adverse Events on Gene Transfer Trials") must be included with each SAE submitted.**

Reporting to FDA:

- Serious adverse events will be forwarded to FDA by the IND Sponsor (Safety Project Manager IND Office) according to 21 CFR 312.32.

It is the responsibility of the PI and the research team to ensure serious adverse events are reported according to the Code of Federal Regulations, Good Clinical Practices, the protocol guidelines, the sponsor's guidelines, and Institutional Review Board policy.

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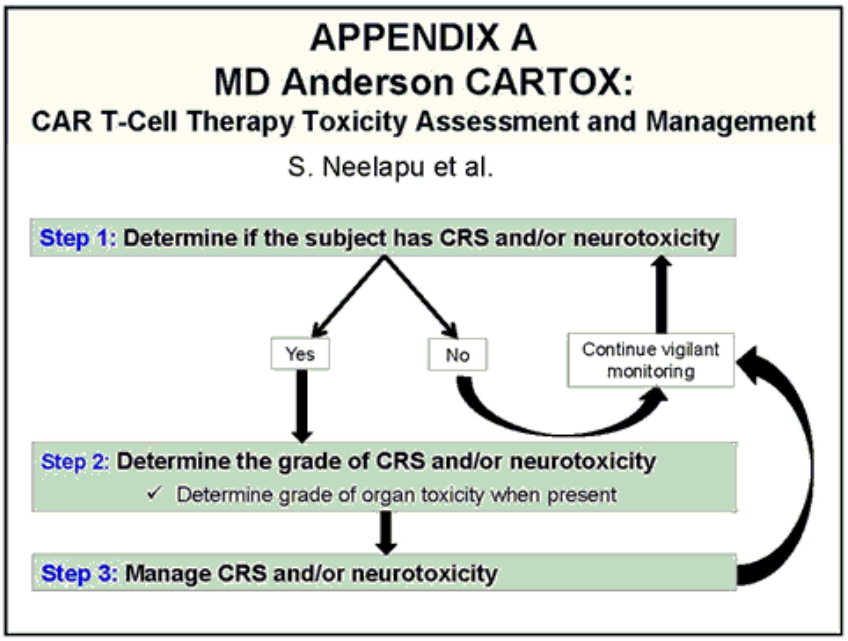
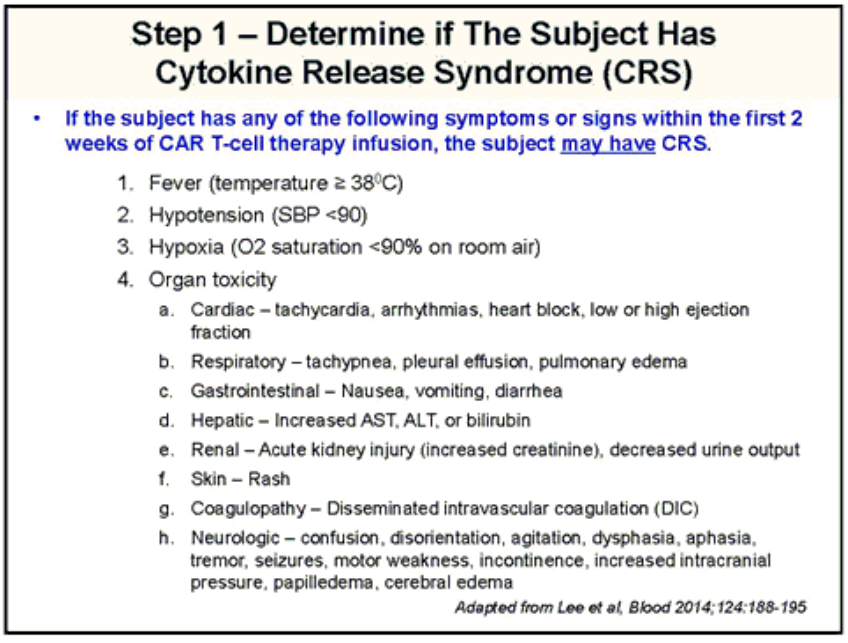
Appendix A. - CARTOX: CAR T-Cell Therapy Toxicity Assessment and Management





Step 2 – Determine the Grade of CRS

- **CRS grade should be determined at least twice daily and any time there is a change in patient's status.**

Category	Symptom/Sign	CRS Grade 1[a]	CRS Grade 2[b]	CRS Grade 3[b]	CRS Grade 4[b]
Vital signs	Temp ≥ 38°C	Yes	Any	Any	Any
	SBP < 90	No	Responds to IV fluids or low-dose vasopressor	Needs high-dose or multiple vasopressors[d]	Life- threatening
	Needing oxygen for O_2 sat >90%	No	FiO2 <40%	FiO2 ≥40%	Needing ventilator support
Organ toxicity[c]	See Step 1	Grade 1	Grade 2	Grade 3 or grade 4 transaminitis	Grade 4 except grade 4 transaminitis

[a] Grade 1 CRS may manifest as fever and/or grade 1 organ toxicity

[b] For Grades 2, 3, or 4 CRS, any one of the criteria other than temperature is sufficient

[c] See Appendix 1 and CTCAE, version 4 for grading of organ toxicity.

[d] See Appendix 2 for definition of high-dose vasopressors

Adapted from Lee et al, Blood 2014;124:188-195

Step 3 – Manage CRS and Organ Toxicity

[a] High risk for severe CRS: Bulky disease, co-morbidities, age ≥60 yrs, early onset CRS (<3 days)

CRS Grade	Symptom or Sign	Management
Grade 1	Fever or Grade 1 Organ Toxicity	• Acetaminophen and hypothermia blanket as needed for fever • Ibuprofen if fever is not controlled with above; use with caution or avoid if thrombocytopenic • Assess for infection with blood and urine cultures, and chest x-ray • Consider antibiotics and filgrastim if neutropenic • IV fluids as needed • Symptomatic management of constitutional symptoms and organ toxicities
Grade 2	Hypotension	• IV fluid bolus of 500 – 1000 mL normal saline • Tocilizumab 8 mg/kg IV q 6h as needed for up to 3 doses / 24h • May give a second IV fluid bolus if SBP remains <90 in 1 hour • If hypotension persists after two fluid boluses, start vasopressors, transfer patient to ICU, and obtain ECHO • In patients at high-risk[a] or if hypotension persists after 1-2 doses of tocilizumab, may use Dexamethasone 10 mg IV q 6h • Manage fever and constitutional symptoms as in Grade 1 CRS
	Hypoxia	• Use supplemental oxygen as needed • Use tocilizumab +/- corticosteroids as in hypotension • Manage fever and constitutional symptoms as in Grade 1 CRS
	Grade 2 Organ Toxicity	• Manage organ toxicity as per standard guidelines • Use tocilizumab +/- corticosteroids as in hypotension • Manage fever and constitutional symptoms as in Grade 1 CRS

Step 3 – Manage CRS and Organ Toxicity (cont.)

CRS Grade	Symptom or Sign	Management
Grade 3	Hypotension	• IV fluid boluses as needed as in Grade 2 CRS • Tocilizumab 8 mg/kg IV q 6h as needed for up to 3 doses / 24h if not administered previously • Use vasopressors as needed • Transfer patient to ICU and obtain ECHO if not done already • **AP1903 (0.4 mg/kg as a 2 h IV infusion). If no improvement in 12 h:** • Start Dexamethasone 10 mg IV q 6h* • Manage fever and constitutional symptoms as in Grade 1 CRS
	Hypoxia	• Use supplemental oxygen as needed • Use tocilizumab + corticosteroids as above • Manage fever and constitutional symptoms as in Grade 1 CRS
	Grade 3 Organ Toxicity or Grade 4 Transaminitis	• Manage organ toxicity as per standard guidelines • Use tocilizumab + corticosteroids as above • Manage fever and constitutional symptoms as in Grade 1 CRS
Grade 4	Hypotension	• Manage as in Grade 3 CRS
	Hypoxia	• Mechanical ventilation
	Grade 4 Organ Toxicity Excluding Transaminitis	• Manage as in Grade 3 CRS

*Methylprednisolone has also been used at doses ranging from 1 mg/kg IV q12 h or 500 mg IV q12 h for 3 days followed by rapid taper at 250 mg q12 h x 2 days, 125 mg q12h x 2 days, and 60 mg q12 h x 2 days). Steroid taper may be individualized depending on toxicity

Step 4 – Determine the CTCAE and MDACC Grade of Neurotoxicity

	Symptom/Sign	Grade 1	Grade 2	Grade 3	Grade 4
CTCAE	Level of Consciousness	Mild drowsiness / sleepiness	Moderate somnolence, limiting instrumental ADL	Obtundation or stupor	Life-threatening needing urgent intervention or mechanical ventilation
	Orientation / Confusion	Mild disorientation / confusion	Moderate disorientation, limiting instrumental ADL	Severe disorientation, limiting self-care ADL	
	ADL / Encephalopathy	Mild limiting of ADL	Limiting instrumental ADL	Limiting self-care ADL	
	Speech	Dysphasia not impairing ability to communicate	Dysphasia with moderate impairment in ability to communicate spontaneously	Severe receptive or expressive dysphasia, impairing ability to read, write or communicate intelligibly	-
	Seizure	Brief partial seizure, no loss of consciousness	Brief generalized seizure	Multiple seizures despite medical intervention	Life-threatening; prolonged repetitive seizures
	Incontinent or Motor Weakness			Bowel / bladder incontinence; Weakness limiting self-care ADL, disabling	
	MDACC 10-Point Neurotoxicity Grade	Mild (7-9)	Moderate (3-6),	Severe (1-2), grade 1 and 2 papilledema with CSF opening pressure (op) < 20 mm Hg	Critical (Obtunded, convulsive status epilepticus; motor weakness, grade 3, 4 & 5 papilledema, CSF op ≥ 20 mm Hg, cerebral edema)

Step 5 – Manage Neurotoxicity

Grade	Management
Grade 1	• Vigilant supportive care; Aspiration precautions • Daily simplified neurologic examination as in Appendix B3 • Fundus exam to document +/- papilledema • MRI brain and diagnostic lumbar puncture with opening pressure (op); MRI spine if focal signs • Daily 30 min EEG; if no seizures on EEG, continue levetiracetam 750 mg q 12 h • If EEG shows non-convulsive status epilepticus, treat as per algorithm in Appendix B4 • Consider Tocilizumab 8 mg/kg IV if associated with Grade 2 or greater CRS
Grade 2	• Manage as per Grade 1 • Consider ICU transfer if associated with Grade 2 or greater CRS • Tocilizumab 8 mg/kg IV if associated with Grade 2 or greater CRS
Grade 3	• Manage as per Grade 1 • Tocilizumab 8 mg/kg IV q 6h for up to 3 doses / 24 h if not administered previously • **AP1903 (0.4 mg/kg as a 2 h IV infusion); If no response in 12 hours:** • Consider corticosteroids (e.g. dexamethasone 10mg IV q6h or methylprednisolone 1 mg/kg IV q 12h) for worsening symptoms despite tocilizumab; Continue steroids until reversal of toxicity and taper over 2 weeks • Low grade (1 & 2) papilledema with CSF op < 20 mm Hg, see Appendix B6 • Consider ICU transfer if associated with Grade 2 or greater CRS • Consider repeat neuro-imaging (CT or MRI) q 2-3 days if persistent neurotoxicity ≥ grade 3
Grade 4	• Manage as per Grade 3 • ICU monitoring • **AP1903 (0.4 mg/kg as a 2 h IV infusion)** • High-dose corticosteroids (e.g. Methylprednisolone IV 1 g/day x 3 days followed by rapid taper at 250 mg q12 h x 2 days, 125 mg q12 h x 2 days, and 60 mg q12 h x 2 days); Continue until reversal of toxicity and taper over 2 weeks • For convulsive status epilepticus, treat as per algorithm in Appendix B5 • High grade (3, 4, & 5) papilledema, CSF op ≥ 20 mm Hg, or cerebral edema, see Appendix B6

Appendix B. - CARTOX: CTCAE Grading of Common Organ Toxicities

Appendix B.1 – CTCAE Grading of Common Organ Toxicities

Category	Symptom/Sign	Grade 1	Grade 2	Grade 3	Grade 4
Cardiac	Sinus Tachycardia	Asymptomatic, No intervention needed	Symptomatic, non-urgent intervention indicated	Urgent intervention indicated	-
	Arrhythmia or Heart Block	Asymptomatic, No intervention needed	Symptomatic, non-urgent intervention indicated	Urgent intervention indicated	Life-threatening
	Ejection Fraction Decreased	-	EF 50-40% or 10-19% drop from baseline	EF 39-20% or >20% drop from baseline	EF <20%
Respiratory	Pleural Effusion	Asymptomatic, No intervention needed	Symptomatic, intervention indicated (diuretics or thoracentesis)	Symptomatic with respiratory distress; needs surgical intervention (chest tube or pleurodesis)	
	Pulmonary Edema	Minimal dyspnea on exertion	Moderate dyspnea one exertion; Medical intervention indicated; limits instrumental ADL	Dyspnea at rest; oxygen indicated; limits self-care ADL	Life-threatening; urgent intervention or ventilatory support indicated

*For toxicities not listed here, please refer to CTCAE, version 4 for grading.

Appendix B.1 – CTCAE Grading of Common Organ Toxicities (cont.)

Category	Symptom/Sign	Grade 1	Grade 2	Grade 3	Grade 4
Gastrointestinal	Nausea	Loss of appetite without alteration in eating habits	Oral intake decreased without dehydration or weight loss	Inadequate oral caloric or fluid intake, tube feeding or TPN	-
	Vomiting	1-2 episodes / 24 hrs	3-5 episodes / 24 hrs	>6 episodes / 24 hrs, tube feeding or TPN	Life-threatening
	Diarrhea	Increase of <4 stools/day over baseline	Increase of 4-6 stools/day over baseline	Increase of ≥7 stools/day over baseline; limits self-care ADL	Life-threatening
Hepatic	AST or ALT	>ULN – 3 x ULN	>3-5 x ULN	>5-20 x ULN	>20 x ULN
	Total Bilirubin	>ULN – 1.5 x ULN	>1.5-3 x ULN	>3-10 x ULN	>10 x ULN
Renal	Urine Output Decreased	-	-	Oliguria (<80 mL / 8 hrs)	Anuria (<240 mL / 24 hrs)
	Acute Kidney Injury	Creatinine 1.5-2 x above baseline	Creatinine 2-3 x above baseline	Creatinine >3 x baseline or > 4 mg/dL	Life-threatening; dialysis indicated
Coagulopathy	DIC	-	Laboratory findings with no bleeding	Laboratory findings with bleeding	Life-threatening; Urgent intervention indicated

*For toxicities not listed here, please refer to CTCAE, version 4 for grading.

Appendix B.2 – Definition of High-Dose Vasopressors

Vasopressor	Definition of High-Dose Vasopressor
Norepinephrine monotherapy	≥ 20 μg/min
Dopamine monotherapy	≥ 10 μg/kg/min
Phenylephrine monotherapy	≥ 200 μg/min
Epinephrine monotherapy	≥ 10 μg/min
If on vasopressin	Vasopressin + norepinephrine equivalent of ≥ 10 μg/min*
Of on combination vasopressors (not vasopressin)	Norepinephrine equivalent of ≥ 20 μg/min*

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

Appendix B.3 – Simplified 10-Point Neurologic Examination

- "Orientation to year, month, city, hospital, President: 5 points
- Ability to write a standard sentence (e.g. National bird is the bald eagle): 1 point
- Name 3 objects (point to clock, pen, button): 3 points
- Count 10 backwards from 100: 1 point

MDACC 10-point Neurotoxicity Grading

- ✓ Normal – score 10
- ✓ Mild neurotoxicity – score 7-9
- ✓ Moderate neurotoxicity – score 3-6,
- ✓ Severe neurotoxicity – score 1-2, mild papilledema (grade 1 and 2) with CSF opening pressure < 20 mm Hg
- ✓ Critical neurotoxicity – Obtunded / stupurous and/or any new motor weakness and/or convulsive status epilepticus, and/or higher grade papilledema (grade 3, 4, and 5), CSF opening pressure ≥ 20 mm Hg, cerebral edema seen on neuro-imaging

Appendix B.4 – Management of Non-Convulsive Status Epilepticus

- Assess ABC/airway positioning/high flow O_2, check blood sugar
- Ativan 0.5 mg IV × 1 with additional 0.5 mg IV q 5 min up to a total of 2 mg to control electrographical seizures
- Levetiracetam 500 mg IV bolus
- If seizures persist, transfer to ICU and add a second agent Phenobarbital loading dose 60 mg IV
- Maintenance doses after resolution of status epilepticus
  - ✓ Ativan 0.5 mg IV q 8 hours × 3 doses
  - ✓ Increase Keppra to 1000 mg IV q 12 h
  - ✓ Phenobarbital 30 mg IV q 12 h

Appendix B.5 – Management of Convulsive Status Epilepticus

- Assess ABC/airway positioning / high flow O2, check blood sugar
- MERIT team to evaluate for transfer to ICU
- Ativan 2 mg IV × 1 with additional 2 mg IV to a total of 4 mg to control electrographic seizures
- Levetiracetam 500 mg IV bolus
- If seizures persist, transfer to ICU and add a second agent Phenobarbital loading dose 15 mg/kg IV
- Maintenance doses after resolution of status epilepticus
  - ✓ Ativan 0.5 mg IV q 8 h × 3 doses
  - ✓ Increase Levetiracetam to 1000 mg IV q 12 h
  - ✓ Phenobarbital 1-3 mg/kg IV q 12 h
  - ✓ Continuous EEG, if seizures are refractory

Appendix B.6 – Management of Cerebral Edema

- **Grade 1 and 2 papilledema with CSF op < 20 mm Hg without cerebral edema**
  - ✓ Acetazolamide (Diamox) 1000 mg IV followed by 250 mg-1000 mg iv q 12 h (adjust dose based on renal and acid/base balance)
- **Grade 3, 4, and 5 papilledema, any cerebral edema on imaging studies, or CSF op ≥ 20 mm Hg**
  - ✓ Use high-dose steroids as per Grade 4 neurotoxicity along with the following measures for management of cerebral edema
  - ✓ Elevate head to 30 degrees
  - ✓ Hyperventilation to achieve target $PaCO_2$ of 28-30 mm Hg for 24 h
  - ✓ Hyperosmolar therapy with either mannitol 20% **or** hypertonic saline (3% or 23.4%)
    - Mannitol: initial dose 0.5-1 g/kg, maintenance at 0.25-1 g/kg every 6 h (metabolic profile and serum osmolality q 6 h, hold mannitol if serum osmolality ≥ 320 mOsm/kg or osmolol gap ≥40)
    - Hypertonic saline: initial 250 mL of 3% hypertonic saline, maintenance at 50-100 mL/h (q 4 h electrolytes, hold infusion if serum Na ≥155 mEq/L)
    - Imminent herniation: Initial 30 mL of 23.4% hypertonic saline (may repeat in 15 min)
  - ✓ If patient has Ommaya reservoir, drain CSF to target op < 20 mm Hg
  - ✓ Consider neurosurgery consultation, IV anesthetics for burst-suppression EEG
  - ✓ Metabolic profile q 6 h, daily Head CT and adjust above medications to prevent rebound cerebral edema, renal failure, electrolyte abnormalities, hypovolemia and hypotension.