Interleukin-15 **a**rmored **G**lypican-3-specific chimeric **a**ntigen **r**eceptor expressing autologous T cells as immunotherapy for children with solid tumors (**AGAR**)

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CHECKLIST FOR PATIENT ELIGIBILITY –PROCUREMENT

PATIENT ID _____ PATIENT NAME _____

YES	NO	VALUE/DATE	INCLUSION CRITERIA
Any "NO" answers will make a patient ineligible for study participation.			
			Diagnosis of GPC3-positive solid tumor
			Age ≥ 1 year and ≤ 21 years
			Life expectancy of ≥ 16 weeks
			Lansky or Karnofsky score ≥ 60%
			Child-Pugh-Turcotte score < 7 (for patients with hepatocellular carcinoma only)
			Barcelona Clinic Liver Cancer Stage A, B or C (for patients with hepatocellular carcinoma only)
			Informed consent explained to, understood by and signed by patient/guardian. Patient/guardian given copy of informed consent
			GPC3 expression (as determined by immunohistochemistry) with an extent score of ≥ Grade 2 (>25% positive tumor cells) and an intensity score of ≥ 2 (scale 0-4).

YES	NO	VALUE/DATE	EXCLUSION CRITERIA
Any "YES" answers will make a patient ineligible for study participation.			
			History of hypersensitivity reactions to murine protein-containing products or presence of human anti-mouse antibody (HAMA) prior to enrollment (only patients who have received prior therapy with murine antibodies)
			History of organ transplantation
			Known HIV positivity
			Active bacterial, fungal or viral infection (except Hepatitis B or Hepatitis C virus infections)
			Actively progressing CNS metastases

Signature of MD _____ Date _____

CHECKLIST FOR PATIENT ELIGIBILITY - TREATMENT

PATIENT ID _____ **PATIENT NAME** _____

YES	NO	VALUE/DATE	INCLUSION CRITERIA
Any "NO" answers will make a patient ineligible for study participation.			
			Age ≥1 year and ≤ 21 years
			Lansky or Karnofsky score ≥ 60%
			Child-Pugh-Turcotte score < 7 (for patients with hepatocellular carcinoma only)
			Barcelona Clinic Liver Cancer Stage A, B or C (for patients with hepatocellular carcinoma only)
			Creatinine clearance as estimated by Cockcroft Gault or Schwartz ≥ 60 ml/min
			Serum AST< 5 times ULN
			Total bilirubin < 3 times ULN for age
			INR ≤ 1.7 (for patients with hepatocellular carcinoma only)
			Absolute neutrophil count > 500/µl
			Platelet count > 25,000/µl (can be transfused but must be at that level prior to treatment)
			Hgb ≥ 7.0 g/dl (can be transfused but must be at that level prior to treatment)
			Pulse oximetry > 90% on room air
			Refractory or relapsed disease after treatment with up-front therapy and at least one salvage treatment cycle
			Recovered from acute toxic effects of all prior t and investigational agents before entering this study
			Sexually active patients must be willing to utilize one of the more effective birth control methods for 3 months after the T-cell infusion.
			Informed consent explained to, understood by and signed by patient/guardian. Patient/guardian given copy of informed consent

Signature of MD _____ **Date** _____

CHECKLIST FOR PATIENT ELIGIBILITY - TREATMENT

PATIENT ID _____ **PATIENT NAME** _____

YES	NO	VALUE/DATE	EXCLUSION CRITERIA
Any "YES" answers will make a patient ineligible for study participation.			
			Pregnancy or lactation (for women at child-bearing age, birth control is required)
			Uncontrolled infection
			Systemic steroid treatment (≥ 0.5 mg prednisone equivalent/kg/day, dose adjustment or discontinuation of medication must occur at least 24hrs prior to CAR T cell infusion)
			Known HIV positivity
			Active bacterial, fungal or viral infection (except Hepatitis B or Hepatitis C virus infections)
			History of organ transplantation
			History of hypersensitivity reactions to murine protein-containing products or presence of human anti-mouse antibody (HAMA) prior to enrollment (only patients who have received prior therapy with murine antibodies)
			Actively progressing CNS metastases

Signature of MD _____ **Date** _____

1.0 Objectives

The overall goal of this Phase 1 clinical trial is to evaluate the safety of autologous glypican-3 specific chimeric antigen expressing T cells co-expressing IL-15 (15.GPC3-CAR T cells) in children with GPC3-positive solid tumors after lymphodepleting chemotherapy.

1.1 Primary Objective

1.1.1 To determine the safety of escalating doses of an intravenous injection of 15.GPC3-CAR T cells in children with GPC3-positive solid tumors after lymphodepleting chemotherapy.

1.2 Secondary Objective

1.2.1 To determine the maximum tolerated dose (MTD) of 15.GPC3-CAR T cells in treating patients with GPC3-positive solid tumors after lymphodepleting chemotherapy.

1.2.2 To assess the anti-tumor effect of infused 15.GPC3-CAR T cells in children with GPC3-positive solid tumors.

1.3 Exploratory Objectives:

1.3.1 To assess the *in vivo* persistence, phenotype and functional activity of infused 15.GPC3-CAR T cells in children with GPC3-positive solid tumors.

2.0 Background and Rationale

2.1 Glypican 3 (GPC3)-positive tumors of children

Relapsed and refractory solid tumors are challenging to treat. Surgical resection is a mainstay of therapy, given conventional cytoreductive treatment with chemo or radiotherapy is associated with significant toxicity and often induces limited antitumor effect. Thus, safe and effective new therapies are needed. GPC3 is an antigen expressed in a heterogeneous group of cancers including hepatoblastoma (HB), hepatocellular carcinoma (HCC), Wilm's tumor, malignant rhabdoid tumor (MRT), yolk sac tumor, rhabdomyosarcoma, undifferentiated embryonal sarcoma of the liver, and liposarcoma [1-8]. Since GPC3 is selectively expressed on these tumors but not on healthy, mature cells, targeting GPC3 may be an effective strategy to eliminate the cancer cells. Importantly, two recent Phase 1 clinical studies showed that targeting GPC3 is safe, well tolerated and, depending on the density of GPC3 expression on tumor cells, can achieve significant antitumor responses in patients with advanced HCC [9, 10].

2.2 Glypican-3

For safe and effective immunotherapeutic approaches tumor restricted expression of the target antigen is necessary. Glypican-3 (GPC3) is one of the six members of the membrane-bound proteoglycans found in mammals [11]. It is an attractive immunotherapeutic target as it is expressed on the majority of HCCs and not expressed on normal hepatocytes after fetal development or on cirrhotic livers [1-8, 12-14]. In HCC, GPC3 promotes tumor growth by stabilization of Wnt in close physical proximity to the cell membrane, thereby increasing Wnt binding to its receptor resulting in increased cell proliferation [1, 15, 16]. GPC3 contains heparan sulfate side chains, a protein chain containing disulfide bonds and is anchored to the cell surface by glycosyl-phosphatidyl-inositol (GPI) [11, 17]. Mutation in GPC3 results in the Simpson-Golabi-Behmel syndrome as its function is critical for cell proliferation during development [18-20].

2.3 Experience targeting glypican-3 with monoclonal antibodies

Two recent early phase clinical trials tested a GPC3 targeting monoclonal antibody (MAb) “GC33” in 33 patients with advanced HCC. GC33 MAb infusions were well tolerated and no dose limiting toxicities were observed. While no patient was cured of their disease, significant antitumor activity was observed that correlated with the GPC3 expression levels in tumors [9, 10]. Thus, GPC3 is a safe target for other means of immunotherapy including genetically engineered T cells redirected to GPC3 by chimeric antigen receptors (CARs).

2.4 Chimeric antigen receptors

One potentially effective immunotherapeutic strategy is to generate tumor-specific effector cells by genetically engineering T cells to express chimeric antigen receptors (CARs), which most commonly consist of an extracellular antigen recognition domain (scFv) from a monoclonal antibody (Mab), a transmembrane domain, and intracellular signaling domains derived from the T-cell receptor CD3-ζ chain and from costimulatory molecules [21]. T cells genetically modified with CARs can combine the specificity of monoclonal antibodies with the active biodistribution and cytotoxic function of effector cells. CAR T cells have numerous advantages over immunotherapies based on monoclonal antibodies or T cells alone [22-24].

1) CARs are MHC-unrestricted, so that tumor escape by downregulation of HLA class I molecules or defects in antigen processing are bypassed.
2) Both $CD4^+$ and $CD8^+$ T cells can express the same CAR, so that the full network of T cell function is directed against tumor cells.
3) CAR-mediated effector function can be effective against cells that are relatively resistant to antibody and complement.
4) Cytokine secretion upon CAR T cell activation by tumor antigen recruits additional components of the immune system, amplifying antitumor immune responses.
5) Unlike intact antibodies, T cells can migrate through microvascular walls, extravasate and penetrate the core of solid tumors to exert their cytolytic activity.
6) A single CAR T-cell can sequentially kill a multiplicity of target cells over a prolonged period of time.

Clinical trials have shown potent antitumor activity of CAR T cells in patients with CD19-positive hematological malignancies, including in patients with significant tumor burden [25-28] In regards to targeting GPC3, GPC3-specific CARs (GPC3-CARs) have been generated by several groups of investigators including ours. GPC3-CAR T cells have potent antitumor activity in preclinical models with GPC3-positive tumors (HCC, lung cancer) [1, 29]. One phase 1 clinical trial with GPC3-CAR T cells was recently completed (NCT02395250), and two are currently in progress at our institution, including one in in pediatrics (NCT02932956) and one in adults (NCT02905188).

This protocol builds on established safety with CAR T cells to redirected to GPC3 by co-expressing IL-15 and iC9 to increase expansion, persistence and antitumor activity; and enable rapid elimination of IL15 co-expressing cells if necessary.

2.5 Extending the Survival of CAR expressing T Cells

Full activation of T cells is accomplished after they bind to a target via their native receptor and receive stimulation from activating ligands on antigen presenting cells (APCs) as well as cytokines secreted in the local microenvironment. Most tumor cells

lack the additional costimulatory ligands needed for enhanced T-cell activation; therefore, 2nd generation CARs have been engineered to include another stimulatory domain [30]. The most common domain added to 2nd generation CARs incorporates the intracytoplasmic portion of CD28 that would normally be activated upon binding to the activating ligands on APCs and this construct has improved the function of adoptively transferred CAR T cells in humans [31]. Other stimulatory domains used include OX40, 41BB, inducible T-cell co-stimulator (ICOS), and DNAX activation protein 10 (DAP10) [23]. Recently, clinical trials testing CD19-specific CARs with 41BB costimulatory endodomain showed breakthrough results in the treatment of CD19-positive malignancies including 91% complete remission rate of patients with relapsed/refractory acute lymphoblastic leukemia [25, 26, 28].

2.6 Generation and preclinical evaluation of GPC3-CAR T cells under study

We systematically evaluated the anti-tumor properties of T cells expressing GPC3-CARs containing CD3zeta (Gz) with CD28 (G28z) or 41BB (GBBz) costimulatory endodomain. We tested T cells expressing Gz, G28z or GBBz against HB, HCC and MRT cell lines in vitro and evaluated their ability to expand, persist and eliminate established human HCC and MRT xenografts in vivo. We show that the inclusion of 4-1BB alone in GPC3-CAR is sufficient to generate T cells with enhanced Th-1-like cytokine response, enhanced proliferation and *in vivo* persistence, and potent therapeutic activity in xenogeneic tumor models of GPC3+ positive solid tumors. Based on these results, the GBBz construct was selected for further development. Next, we evaluated if T cells expressing GBBz and IL-15 in combination have superior antitumor properties compared GBBz alone. We determined that GBBz T cells co-expressing IL-15 specifically and effectively kill GPC3-positive tumor cells in an antigen-dependent manner. We also showed that GPC3-CAR T cells co-expressing IL-15 exhibit enhanced persistence *in vivo* and mediate superior tumor control and survival of tumor-bearing mice. These results provide a strong rationale to evaluate IL-15 co-expressing GPC3 CAR T cells in patients with liver tumors in the second cohort of this clinical study.

2.7. Inducible caspase 9 (iC9) suicide gene

The use of T cells containing an inducible caspase 9 (iC9) suicide gene have the potential to limit toxicities, such as CRS and neurotoxicity that do not respond to current standard of care interventions like steroids and IL-1 and IL-6 inhibitor, and have been evaluated *in vitro* and in clinical trials. Briefly, iC9-mediated suicide is based on conditional dimerization of pro-apoptotic molecules, that are constructed from human proteins and therefore less likely to be immunogenic [32] iC9s generated by joining a drug-binding domain to human caspase 9. The drug-binding domain consists of human FK506-binding protein (FKBP12) with an F36V mutation. This point mutation increases the binding affinity of FKBP12 to non-toxic synthetic homodimerizers, AP20187 or AP1903 (rimiducid) [33, 34]. Administration of AP20187 or rimiducid dimerizes and activates caspase 9; this activates downstream caspases, leading to apoptosis within 24 hours. In patients receiving stem cell transplantation and donor-derived T cells expressing iC9, treatment with rimiducid effectively eliminated 99% of T cells and symptoms of graft-versus-host disease after a single infusion and repeat infusions could eliminate residual T cells if necessary [35-37]. These studies provide evidence that the rimiducid mediated iC9 activation is a safe and effective approach to transduced T cells.

The iC9 genetic modification, unlike other suicide gene systems such as the HSV-TK based suicide gene, is human derived and therefore likely to be less immunogenic. Previous studies have shown that T-cells genetically modified with iC9 suicide gene construct were detected in peripheral blood and increased in number over time, despite their constitutive transgene expression. Moreover, pre-clinical and clinical studies show that killing occurs with much greater rapidity (within 3 hours) than other suicide gene systems. Further advantages of the iC9 system are that killing induced by rimiducid, is primarily restricted to activated/proliferating cells, thus preferentially targeting activated T cells, and as shown in preclinical studies, furthermore the extent of killing efficacy can be modulated through a wide range of Rimiducid concentration eliminating only a fraction at lower and close to complete elimination of iC9 expression cells at higher concentration [38].

In two previous clinical trials, patients who developed Graft Versus Host Disease (GVHD) after infusion of allogeneic iC9 gene-modified T-cells, were given a single dose of dimerizing drug, rimiducid. This led to elimination of more than 90% of the iC9-gene modified T cells within 30 minutes after administration and a further log reduction within 24 hours without any reports of infusion related toxicity. The residual iC9 gene modified T cell population expanded over the next 4-14 days and continued to help repopulate the subjects' immune system without recurrence of GVHD. When examined ex vivo, these non-alloreactive iC9 T cells remained susceptible to apoptosis following exposure to the dimerizer rimiducid. The iC9 gene is most highly expressed in activated and proliferating T cells and if cells are not activated, expression of the transgene is lower and iC9 levels are insufficient to induce apoptosis of the entire population after single rimiducid dose exposure. This hypothesis was supported by the CASPALLO [35] and DOTTI [37] clinical trials showing activation- dependent induction of iC9 in T lymphocytes and enhanced susceptibility to dimerizer drug in activated versus resting T cells.

T cells in this protocol will be co-transduced with two retroviral vectors: GPC3-CAR (SFG.GPC3.4-1BB.z) and iC9.NGFR.IL15 (SFG.iC9.2A.NGFR.2A.IL-15.). This strategy will therefore allow for the preferential killing of the CAR T cells with the highest potency, thereby acting as a safety switch in the setting of toxicities.

2.8 Rationale for lymphodepletion before adoptive T-cell transfer

Lymphodepletion has been shown to improve the anti-tumor efficacy of adoptive cell therapies by direct antineoplastic effect of the used chemotherapeutic agents and by improving survival and function of adoptively transferred cells [39]. The size of the lymphoid compartment is tightly controlled by homeostatic factors, including the access to antigen-presenting cells (APCs), major histocompatibility complex (MHC) presenting self and antigenic peptides, and direct influence of host T lymphocytes including regulatory cells CD4+CD25+FoxP3+ Treg, as well as the competition for cytokines such as IL-2, IL-7, IL-15 and IL-21 [40-48]. Administration of certain lymphodepleting chemotherapy or total body irradiation, reduces the patient's lymphoid cell pool, improves the adoptively transferred cells access to APCs, peptides presented by MHCs and homeostatic cytokines resulting in superior expansion and persistence of transferred cells [39, 49, 50]. Most importantly, after lymphodepletion, long term complete remissions are achievable in heavily pretreated cancer patients with genetically engineered adoptive cell therapies [25, 27, 51]. The most commonly used lymphodepleting regimens combine cyclophosphamide and fludarabine (cy/flu)

and a fractionated cy/flu regimen was used in the only clinical protocol to date resulting in sustained complete remission in a patient with bulky disease [39, 51, 52]. Therefore, we will use a fractionated cy/flu regimen for lymphodepletion in this trial.

2.9 Rationale for the current study

Patients with relapsed / refractory GPC3+ tumors are in urgent need for novel therapies. We systematically tested T cells expressing distinct GPC3-CARs in preclinical models of GPC3+ solid tumor models and found robust antitumor activity against these tumors by GBBz which can be further enhance by IL-15 co-expression in GBBz T cells [53, 54]. We hypothesize that autologous T cells expressing a 15.GPC3-CAR will be safe and we further hypothesize that these cells will have antitumor activity in patients with relapsed/ refractory GPC3+ tumors. We will conduct a dose escalation Phase 1 clinical trial to test our hypotheses.

2.10. Rationale for CAR T cell dose:

Our group has treated three children with GPC3-CAR T cells at the dose level of $1x10^7/m^2$ dose (NCT02932956). The infusions were well tolerated and dose limiting toxicities were not detected. We are enrolling on the $3x10^7/m^2$ dose level currently. A recent publication showed T cells expressing a 3rd generation GPC3-CAR to be safe when total of 13 adult patients with hepatocellular carcinoma received a median of 19.9×10^8 cells [55]. We expect that enrollment on the $3x10^7/m^2$ GPC3-CAR T cells will be safe and we will subsequently start enrollment on the $3x10^7/m^2$ dose level with 15.GPC3-CAR T cells. If safety is not confirmed on this dose level with GPC3-CAR T cells, we will start enrollment on $1x10^7/m^2$ dose with 15.GPC3-CAR T cells.

3.0 Risks of this study

Potential toxicities may be categorized as those related to: i) infusion of T cells; ii) general consequences of retroviral transduction; iii) specific consequences of the transgene expressed (targeting GPC3, cytokine release syndrome, neurotoxicity); and iv) risks of using fludarabine and cyclophosphamide.

3.1 Infusion of CAR T cells

In this study, we will be administering transduced autologous peripheral blood activated T cells modified to express a CAR directed against GPC3 and expectedly the infusion product will contain some percentage of non-transduced T cells. Many previous studies have infused larger numbers of ex-vivo cultured T cells that have been activated ex vivo, with no adverse effects [56, 57]. Thus, the risk from infusing autologous T cells will be minimal.

3.2 General Consequences of Retroviral Transduction

Retroviral transduction results in new, random integrations in host cell DNA, which rarely may cause abnormal or uncontrolled proliferation. This effect is much more common with replication-competent retrovirus (RCR) where each cell receives multiple integrants. Several publications have now reviewed extensive and long-term data on RCR monitoring in T cells immediately after gene modification with retroviral and in subsequent follow-up samples from patients; no evidence for RCR has yet been found, providing important evidence for safety[58-60].

Genotoxicity with retroviral vectors was seen in the initial trials transferring retroviral vectors to hemopoietic stem cells to correct inherited immunodeficiencies in the late 1990s and early 2000s. These studies demonstrated clinical activity in patients, but several years after treatment, some patients in the SCID trials, as well as patients treated for chronic granulomatous disease and Wiskott-Aldrich syndrome, developed acute leukemias due to activation of proto-oncogenes adjacent to proviral insertions[61-

63]. These serious toxicities led to adoption of enhancer-deleted lentiviral or retroviral vectors for HSC clinical gene therapies. Up to now, these newer vectors have produced disease amelioration without genotoxicity[64].
Our proposal should have a very different risk profile as we are transferring retroviral vectors into T cells. Although the inclusion of the 41BB endodomain in the CAR may enhance proliferation of transgenic T cells in response to the GPC3 antigen, there is no reason to believe this effect will lead to uncontrolled T-cell expansion. *Ex vivo* and published *in vivo* data using several artificial T-cell receptors shows that 41BB sustains limited and temporary expansion over approximately 3-6 weeks [65, 66]. Thereafter, the cells maintain the expression of the transgene, but proliferate further only in the presence of both antigen and exogenous cytokines. Our recent clinical trials using CAR-T cells including the 41BB endodomain also indicate that these T cells expand significantly for only 2-3 weeks and then they decline without evidence of further expansion [67, 68].

To date more than 1000 patients have received genetically modified T cells in clinical trials, including patients we have treated on our protocols using T cells modified with gammaretroviruses [67-72]. In none of these, nor in clinical trials outside our center, has malignancy caused by retroviral transduction been reported.

Patients eligible for the current study will have relapsed / refractory GPC3-positive solid tumors. In light of this, the natural history and poor prognosis of these malignancies, and given the entire previous experience with retroviral gene therapy, we feel that the small risks of retrovirally-induced leukemogenesis are small and are justified in these patients.

3.3 Specific consequences of transgenes expressed

Specific consequences of the transgene expressed includes targeting GPC3, the use of IL-15 and CRS.

3.3.1 Targeting GPC3 with CAR T cells

No clinical trial results have been reported in the literature to date from testing GPC3-CAR T cells in patients; thus GPC3-specific side effects are not known. Expression of GPC3 is highly specific and restricted to malignant tissues (1, 2, 5, 12, 13). While Baumhoer *et al* reported some expression of GPC3 on gastric glands (3/7 samples [43%]), kidney tubules (9/17 samples [53%]), and testicular germ cells (2/16 samples [13%]) (4), this was not confirmed by Gao *et al* (1). MAb- based GPC3 targeting in 2 independent Phase 1 clinical trials was safe, and no dose limiting toxicities were observed in 33 HCC patients as discussed in Section 2.3. Mild gastrointestinal toxicities were described (nausea/vomiting/diarrhea), but no nephritis or testicular inflammation was detected. Should 15.GPC3-CAR T cells produce direct toxicity against GPC3-expressing normal tissues, a number of therapeutic maneuvers are available. We will first use rimiducid (the chemical inducer of dimerization) (see Section 6.3.8). Alternatively, corticosteroids in doses used to treat graft-versus host-disease (GvHD) or anti-T-cell antibodies such as Campath 1H can be used, as both of which will deplete the majority of circulating transduced cells.

3.3.2 Co-expression of IL-15

There is prior safety experience using IL-15 in patients. Conlon et al. examined the safety of recombinant IL-15 in eighteen patients with melanoma and RCC. Doses ranged between 0.3-3 µg/kg/day given on twelve consecutive days intravenously. Peak IL-15 concentrations were 1260 (+/- 348) pg/ml for 0.3 µg/kg/day dosing. DLTs included thrombocytopenia, hypotension and transaminase elevations. The maximum

tolerated dose was defined at 0.3 µg/kg/day dose. Stable disease was the best response per RECIST criteria, five patients had 10-30% decrease in marked lesions with two of these patients clearing their respective lung lesions [73].

Based on these results, IL-15 can be administered safely to patients with cancer. Peak concentrations in peripheral blood of these patients were high. In our *in vivo* animal studies, systemic levels of IL-15 at the time of peak CAR T cell expansion were detected at levels similar to control T cells at 3 - 4 log lower concentrations compared to the human studies using IL-15 bolus injections [73, 74]. IL-15 produced by GPC3-CAR T cells will likely only locally produce effects on the adoptively transferred CAR T cells and bystander cells. Should 15.GPC3-CAR T cells induce toxicity of grade 2 (grade 3 respiratory toxicity) or higher, we will consider rimiducid (≤ 50 kg: starting dose of 0.02 mg then escalate to 0.004 mg/kg/dose, 0.04 mg/kg/dose and 0.4 mg/kg/dose if needed; > 50 kg: starting dose of 0.0004 mg/kg then escalate to 0.004 mg/kg/dose, 0.04 mg/kg/dose and 0.4 mg/kg/dose if needed based on response after 6-8 hours of observation following each dose) (see below in Section 6.3.8). We will also consider using steroids (1-2 mg/kg/day of methylprednisolone or equivalent) if suboptimal response to rimiducid. Alternatively, we will consider the JAK1 inhibitor, Ruxolitinib (5 mg PO twice daily for children ≥ 25 kg or 2.5 mg twice daily if <25 kg) as it disrupts JAK1 mediated downstream signaling of IL15; and we found that ruxolitinib can temporarily halt the CAR T cell function.

3.4 Cytokine Release Syndrome and Neurotoxicity

There have been several reported SAEs associated with cytokine release syndrome (CRS) in patients who received T cells [75] or bispecfic T-cell engager molecules [76]. The majority of CRS have been reported after the infusion of CAR T cells [27, 28, 77], but CRS can also occur after the infusion of conventional antigen-specific T cells [78] or tumor infiltrating lymphocytes [79]. Patients will be monitored closely as per study calendar and assessed for evidence of incipient CRS (onset of fever, malaise and dyspnea) and treated promptly. Management of CRS will follow published guidelines [75, 80], and is described in more detail in SOP F 05.11.XX and includes treatment options based on the clinical severity of the symptoms, such as oxygen, inotropic agents, and IL-6 receptor antibody (4-8 mg/kg). If any grade 2 CRS or above not responsive to the aforementioned agents, then rimiducid (see below in 6.3.8) will be administered. Alternatively, TNF-α antibody (5-10 mg/kg) may be given. If resistant to the aforementioned modalities, steroids (1-2 mg/kg/day of methylprednisolone or equivalent) will be administered. The JAK1 inhibitor, Ruxolitinib (5 mg PO twice daily for children ≥ 25 kg or 2.5 mg twice daily if <25 kg) may be considered for cases resistant to above reagents as it disrupts JAK1 mediated downstream signaling of IL15.

There have been several reports of neurotoxicity (e.g. encephalopathy, somnolence, aphasia) after the infusion of CD19-specific CAR T cells [27, 28, 77]. Patients will be monitored closely as per study calendar and assessed for evidence of incipient neurotoxicity (mild somnolence, drowsiness or sleepiness) and treated promptly. Management will include IL-6 receptor antibody (4-8 mg/kg), rimiducid, and if resistant to other modalities, steroids (1-2 mg/kg/day of methylprednisolone or equivalent), as well as prophylactic anti-epileptics as described in the literature [27, 75, 80].

3.5 Lymphodepletion related toxicities

In this protocol we will use fractionated cyclophosphamide and fludarabine for lymphodepletion. This has been a widely used lymphodepletion protocol prior to CAR T-cell infusion [39, 49, 81]. Both cyclophosphamide and fludarabine are commercially available and a list of their toxicities is attached in Appendix V.

3.6 Safety of the synthetic homodimerizer and the inducible Caspase 9 (iC9) safety switch in the IL15-encoding vector (iC9.NGFR.IL-15):

No known risks have been associated with the synthetic homodimerizer rimiducid, which has been evaluated in the United Kingdom and has successfully completed a phase I clinical safety study when rimiducid was administered over a 0.01mg/kg to 1.0mg/kg dose range [34]. The maximal plasma level attained over this dose range was 10 to 1275ng/ml rimiducid (equivalent to 7 to 892nM). rimiducid will be infused as described in **Section 6.3.8**.

4.0 Generation of Transduced Cells

4.1 Blood collection

Up to 90 ml (max of 3ml/kg/day) of peripheral blood will be collected over 24 hours from a given patient. This may be repeated a second time at least 24 hours later, with the limitation not to exceed 180 ml total over the 2 separate collections. The blood is used to generate the cellular components listed below, and for infectious disease testing and HLA typing.

4.2 Retroviral production

A producer line for a gamma-retrovirus encoding the GPC3-CAR and the iC9.NGFR.IL15 has been generated and vector lots have been prepared under cGMP conditions for these patients. The producer cells have been tested for replication competent retrovirus and contamination by bacteria, fungi and mycoplasma and by other viruses as per our certificates of analysis. The harvested supernatants have been filtered, aliquoted and rapidly frozen and stored at –80C. The retroviral supernatants are tested for sterility and endotoxin and to exclude replication competent retrovirus (RCR). We have issued a certificate of analysis in accordance with our SOPs for both vectors.

4.3 CAR T cell production, transduction, and expansion

Briefly, isolated PBMCs will be activated with clinical grade CD3 and CD28 MAbs, transduced with the GPC3-CAR and the iC9.NGFR.IL15 retroviral vector and expanded using cytokines as per SOP D03.53.XX.

4.4 Testing

Products that meet study specific release criteria, as detailed on the Certificate of Analysis (CofA), will be infused as per Section 6.0.

If a positive sterility testing result is reported after the product was infused, the FDA and other relevant parties would be notified as per our manufacturing SOP B01.03.XX (Product Quality Assurance Program and Release and Return of Clinical GMP/GTP Products) and our clinical research SOP J02.06.XX (Serious Adverse Experience and Unanticipated Problem Reporting). Our management of such a situation is further described in our SOP F05.09.XX (Management of Culture Positive Cell Therapy Products).

5.0 Eligibility Criteria

5.1 Procurement Eligibility

5.1.1 Inclusion Criteria:

- Diagnosis of GPC3-positive* solid tumors (as determined by immunohistochemistry with an extent score of >=Grade 2 [>25% positive tumor cells] and an intensity score of >= 2 [scale 0-4]).
- Age ≥1 year and ≤ 21 years
- Lansky or Karnofsky score ≥60% (See Appendix I)
- Life expectancy ≥16 weeks
- Barcelona Clinic Liver Cancer Stage A, B or C (for patients with hepatocellular carcinoma only, See Appendix II)
- Child-Pugh-Turcotte score <7 (for patients with hepatocellular carcinoma only, See Appendix III)
- Informed consent explained to, understood by and signed by patient/guardian. Patient/guardian given copy of informed consent

* GPC3 expression will be evaluated by standard immunohistochemistry (IHC) at Texas Children's Hospital/Baylor College of Medicine, Department of Pathology for all patients to meet procurement eligibility. All patients will send at least 5 unstained slides.

5.1.2. Exclusion Criteria

- History of hypersensitivity reactions to murine protein-containing products OR presence of human anti-mouse antibody (HAMA) prior to enrollment (only patients who have received prior therapy with murine antibodies).
- History of organ transplantation
- Known HIV positivity
- Active bacterial, fungal or viral infection (except Hepatitis B or Hepatitis C virus infections)
- Actively progressing CNS metastases

5.2 Treatment Eligibility

5.2.1 Inclusion Criteria

- Age ≥ 1 year and ≤ 21 years
- Barcelona Clinic Liver Cancer Stage A, B or C (for patients with hepatocellular carcinoma only, See Appendix II)
- Lansky or Karnofsky score ≥ 60% (See Appendix I)
- Child-Pugh-Turcotte score < 7 (for patients with hepatocellular carcinoma only, See Appendix III)
- Adequate organ function:
    - Creatinine clearance as estimated by Cockcroft Gault or Schwartz ≥ 60 ml/min
    - serum AST< 5 times ULN
    - total bilirubin < 3 times ULN for age
    - INR ≤1.7 (for patients with hepatocellular carcinoma only)
    - absolute neutrophil count > 500/µl
    - Platelet count > 25,000/µl (can be transfused but must be at that level prior to treatment)
    - Hgb ≥ 7.0 g/dl (can be transfused but must be at that level prior to treatment)

- Pulse oximetry >90% on room air
- Refractory or relapsed disease after treatment with up- front therapy and at least one salvage treatment cycle
- Recovered from acute toxic effects of all prior chemotherapy and investigational agents before entering this study
- Sexually active patients must be willing to utilize one of the more effective birth control methods for 3 months after the T-cell infusion.
- Informed consent explained to, understood by and signed by patient/guardian. Patient/guardian given copy of informed consent

5.2.2 Exclusion Criteria

- Pregnancy or lactation
- Uncontrolled infection
- Systemic steroid treatment (≥ 0.5 mg prednisone equivalent/kg/day, dose adjustment or discontinuation of medication must occur at least 24hrs prior to CAR T cell infusion)
- Known HIV positivity
- Active bacterial, fungal or viral infection (except Hepatitis B or Hepatitis C virus infections)
- History of organ transplantation
- History of hypersensitivity reactions to murine protein-containing products OR presence of human anti-mouse antibody (HAMA) prior to enrollment (only patients who have received prior therapy with murine antibodies)
- Actively progressing CNS metastases

6.0 Treatment Plan

In this Phase 1 clinical study, patients will receive lymphodepleting chemotherapy (Appendix IV) followed by infusion of 15.GPC3-CAR T cells.

6.1 Pre-infusion lymphodepletion

Patients will receive 3 daily doses of cyclophosphamide together with fludarabine to induce lymphopenia, finishing at least 48 hours before CAR T cell infusion. Cyclophosphamide will be given at a dose of 500 mg/m^2/dose followed by Fludarabine 30 mg/m^2/dose. Infusions should be given following hospital/pharmacy recommendations however at a minimum the cyclophosphamide should be infused over 1 hour and the fludarabine should be infused over 30 minutes. Mesna, IV hydration and anti-emetics should also be provided following local institutional guidelines. T-cell infusion will be given 48 to 96 hours post completion of chemotherapy.

Day	Agent	Dose/Administration
-4	Cyclophosphamide/Fludarabine	500mg/m^2/dose IV / 30mg/m^2/dose IV
-3	Cyclophosphamide/Fludarabine	500mg/m^2/dose IV / 30mg/m^2/dose IV
-2	Cyclophosphamide/Fludarabine	500mg/m^2/dose IV / 30mg/m^2/dose IV
-1		
0 to +2	15.GPC3-CAR T cells	Dose level 1, 2, 3, or 4 as specified below.

6.2 Dose levels and schedule

Four dose levels (DL) will be evaluated. Three to six patients will be assessed on DL (see Section 8).

The following dose levels will be evaluated:

DL1: $3x10^7/m^2$

DL2: $1x10^8/m^2$

DL3: $3x10^8/m^2$

DL4: $1x10^9/m^2$

If dose de-escalation is required on DL1, patients will be enrolled on DL0 at $1x10^7/m^2$ dose. If further dose de-escalation is necessary, half log decreases will be used on DL-1 and on DL-2.

The first patient on each dose level has to be 14 days post T-cell infusion before the second patient can be enrolled.

The doses are calculated according to the actual number of GPC3-CAR transduced T cells.

- Patients may be premedicated with Benadryl 0.1-0.5 mg/kg IV (max 25mg) and Tylenol 10mg/kg po (max 650mg).
- Cell Administration: 15.GPC3-CAR T cells at an expected concentration of $1x10^7$ cells/ml (volume of 2 – 200 ml) will be given by intravenous infusion over up to 5-10 minutes through either a peripheral or a central line.
- Monitoring will follow institutional standards for administration of blood products with the exception that the infusion will be given by a physician.
- Patients will receive supportive care for acute or chronic toxicity. Such care will include blood components or antibiotics and other interventions as appropriate.
- All treatments will be given at the Center for Cell and Gene Therapy at Texas Children's Hospital.
- Patients should not receive other treatment for their cancer for at least 4 weeks post T-cell infusion (for purposes of evaluation). If a patient receives other treatment for their cancer during the first 4 weeks post T-cell infusion, he/she is not evaluable and will need to be replaced.

6.3 Supportive care

6.3.1 Venous Access

Chemotherapy and T cells can be given through peripheral or central intravenous access.

6.3.2 Antiviral prophylaxis

Patients with chronic hepatitis B virus (HBV) infection must receive antiviral prophylaxis (e.g. entecavir) starting 2 weeks prior to CAR T cell infusion until at least 6 weeks post-infusion to prevent viral reactivation.

6.3.3 Allergic Reactions

Standard medications (hydrocortisone, diphenhydramine, and epinephrine)

to treat allergic reactions should be available at the bedside prior to T-cell infusions.

6.3.4 Fever and Neutropenia

Standard of care should be administered for complications resulting from neutropenia.

6.3.5 Blood Products

Blood products should be given when appropriate.

6.3.6. Concomitant Therapy

No other cancer chemotherapy or immunomodulating agents should be used.

6.3.7. Cytokine Release Syndrome, Neurotoxicity and Other Toxicity

6.3.7.1 CRS, management will follow published guidelines, and is described in more detail in **SOP F 05.11.XX** which includes treatment options based on the clinical severity of the symptoms, such as oxygen, steroids and IL-6 receptor antibody (4-8 mg/kg).

6.3.7.2 For CRS persistent despite standard therapy or other Grade 2 (grade 3 respiratory toxicity) toxicities possibly related to 15.GPC3-CAR T cells, we will plan to administer Rimiducid. Dosing will be as follows:

For Weight ≤ 50 kg:

1) Start at 0.02mg/dose.
2) Dose may be escalated to 0.004mg/kg/dose and then a maximum dose of 0.4mg/kg/dose may be given based on the clinical condition of the patient after 6-8 hours of monitoring following each administration.

For weight >50 kg:

1) Start at dose of 0.0004 mg/kg/dose
2) Dose may be escalated to 0.004 mg/kg/dose, 0.04 mg/kg/dose and 0.4 mg/kg/dose if needed based on the clinical condition of the patient after 6-8 hours of monitoring following each administration.

6.3.7.3 If suboptimal response to rimiducid, we will consider the JAK1 inhibitor, Ruxolitinib (5 mg PO twice daily for children ≥ 25 kg or 2.5 mg twice daily if <25 kg) as it disrupts JAK1 mediated downstream signaling of IL15; and we found that ruxolitinib can temporarily halt the CAR T cell function

6.3.8. Use of rimiducid, chemical inducer of dimerization

Packaging and Formulation: The rimiducid for Injection is packaged in a 3 mL Type 1 clear glass serum vials. The content of each vial is composed of the labeled content 10mg (2mL) of rimiducid drug substance dissolved in a sterile, endotoxin free, 25% Solutol HS 15/Water for Injection solution at a rimiducid concentration of 5 mg/mL and at pH 5.0 – 7.5. Each vial is stoppered with a Teflon© coated serum stopper and a flip-off seal. Rimiducid is manufactured and provided by Bellicum Pharmaceuticals.

Labeling: The primary product label (applied directly to the vial) for the Rimiducid for Injection will contain the following information: product name, Rimiducid for Injection; the manufacturer's lot number; product concentration, 5 mg/mL; volume of solution available in the vial; total rimiducid contents of the vial (10mg); a statement, "For IV Administration, contains no preservatives" and the IND notation,

"Caution: New Drug-Limited by Federal Law to Investigational Use".

Storage: The Rimiducid for Injection vials must be stored at 5°C ± 3°C (41°F ± 5°F) in a limited access, qualified refrigerator, preferably without light.

Preparation for Treatment and dosing escalation: Rimiducid will be diluted prior to administration. The rimiducid is administered via IV infusion at the target dose diluted in normal saline with volume as appropriate for weight. Rimiducid will be given at escalating doses starting with 0.0004 mg/kg/dose, 0.04mg/kg/dose and 0.4mg/kg/dose. Dose increase may be given following 6-8 hours of observation based on the indications below:

Indications:

1) Any Grade 2 or greater CRS and neurotoxicity at least possibly related to IL15 expressing T cells.
3) Amy Grade 2 or greater non-CRS related toxicities at least possibly related to IL15 expressing T cells that do not respond to standard of care.

Prescribers: Authorized prescribers of rimiducid are Principal Investigators and IND holders - Dr. Malcolm Brenner and Dr. Helen Heslop, and the lab medical director.

Laboratory tests after rimiducid administration: Peripheral blood samples will be collected immediately prior to administration of rimiducid, 4-8hr, and 72hrs post-rimiducid dose and will be evaluated with FACS and RT-PCR to confirm elimination of iC9 expressing cells. Additional time-points may be collected based on the clinical condition of the patient.

7.0 Patient Evaluation

A complete history, physical examination, and performance status is necessary prior to lymphodepletion (week -1) and prior to T-cell administration. A complete history, physical exam and performance status will be collected at week 1, 2, 3, and 4. A complete history will continue to be collected at week 8, every three months (starting at month 3) for a year, every 6 months for 4 years, and then yearly for a total of 15 years. After week 4, follow up visits may be conducted at the subject's local pediatric oncologist's office with appropriate documentation provided to the research team.

7.1 Study Calendar

The detailed schedules for clinical monitoring are included below.

Study													
	Wk -1^	Wk 0	Wk 1	Wk 2	Wk 3	Wk 4	Wk 8	Mth 3	Mth 6	Mth 9	Mth 12	Every 6 mths x 4 years	Annually x 15 years (total)
Ctx + Flu	X												
CAR T-cell Infusion		X											
Hx^	X	X	X	X		X	X	X	X	X	X	X	X
PE^	X	X	X	X		X							
Performance Status	X	X	X	X		X							
CBC with diff	X	X	X	X	X	X		X	X	X	X		
Lytes, BUN, Cr	X	X	X	X	X	X							
AST, Total Bili, Alb	X	X	X	X	X	X							
AFP@	X	X	X	X	X	X							
HBV-PCR@@	X	X		X		X							
Pregnancy Test (Child Bearing Potential)	X												
Function and Persistence Studies###	X	X*	X**	X	X	X	X	X	X	X	X	X	X
RCR Testing/ Archiving###		X						X	X		X	X***	X***
Cytokines/ Chemokine###	X	X	X**	X	X	X	X	X					
Serum stored for HAMA###		X				X							
Imaging	X					X~							
Tumor biopsy				X$, ###									

Ctx + Flu: Cyclophosphamide and fludarabine ^starts day -4; *pre-infusion and 1 to 4 hours post infusion; **one sample will be drawn 1 week post infusion, a 2nd sample 3-4 days post infusion is optional; ###study specific tests; ***will be done annually; Pregnancy testing is only required in female patients of childbearing potential; ~Post infusion imaging may be performed from 4-6 weeks post infusion with choice of tests dependent on the previous tests that have best estimated the amount of disease.@: Patients with AFP producing tumors only. @@ Patients with HBV infection only.; $At 2-4 weeks post-CAR T cell infusion tumor biopsies will be obtained. ^After week 4, follow up visits may be conducted at the subject's local physician's office with appropriate documentation provided to the research team. Monthly visits can be done within +/- 1 month; weekly visits can be done within +/- one week of the scheduled visit. Must be completed prior to lymphodepletion and prior to T-cell administration.

7.2 Routine Laboratory Investigation

The following routine laboratory investigations will be obtained as outlined in the study calendar:

- CBC and differential: pre-chemotherapy, pre-infusion, at 1, 2, 3 and 4 weeks post T-cell infusion (each weekly visit can be within + and then at 3, 6, 9, and 12 months.
- BUN, Creatinine, Na^+, K^+, Cl^-, CO_2: pre-chemotherapy, pre-infusion, at 1, 2, 3 and 4 weeks post T-cell infusion
- Bilirubin, Albumin, AST: pre-chemotherapy, pre-infusion, at 1, 2, 3 and 4 weeks post T-cell infusion
- AFP (for patients with AFP producing tumors only): pre-chemotherapy, pre-infusion, at 1, 2, 3 and 4 weeks post T-cell infusion
- HBV-PCR (for patients with HBV infection only): pre-chemotherapy, pre-infusion, at 2, and 4 weeks post T-cell infusion
- Pregnancy test will be performed within the week preceding starting chemotherapy on female patients of childbearing potential unless they have no possibility of pregnancy (for example post hysterectomy)
- Other tests: Infectious disease testing will be done at the time of blood procurement for T-cell production; in addition, HLA typing (via blood or buccal swab) will be performed to ensure autologous identity of T-cell product.
- Additional routine laboratory investigations may be performed as per institutional guidelines during the administration of cyclophosphamide and fludarabine.

**for the weekly visits immediately following infusion (1,2,3, and 4 weeks), these can be done within +/- one week of the scheduled visit; Monthly visits can be done within +/- 1 month from the scheduled visit.

7.3 Diagnostic imaging

Imaging studies are required pre-lymphodepleting chemotherapy (within 4 weeks) and 6 weeks (range 4-6 weeks) following T-cell infusion. Contrast enhanced computer tomography (CT) or magnetic resonance imaging (MRI) are acceptable modalities (for patients with liver tumors, arterial and portal venous phase sequences are necessary). If diagnostic imaging studies are performed at other times either during or after treatment on this study, that data will be collected, and information gained will be used for this study. The same modality will be used at the time of enrollment for treatment and response evaluation.

7.4 Tests of T-cell Function and Persistence

The following samples will be obtained pre-chemotherapy, pre-T-cell infusion, 1 to 4 hours post T-cell infusion, 3-4 days post T-cell infusion (optional depending on patient preference), at 1, 2, 3, 4 and 8 weeks, then at 3, 6, 9, and 12 months then at least every 6 months for the first 5 years and then yearly thereafter for the next 10 years. The following analysis will be performed on these peripheral blood samples to monitor function, persistence and safety of transduced T-cells at time-points indicated in the study calendar.

- Quantitative real-time PCR for GPC3-CAR and iC9.NGFR.IL15 construct.
- Immunostaining for GPC3-CAR and NGFR (encoded in the iC9.NGFR.IL15) if CAR PCR is greater than 0.5%
- If detection of CAR T cells is greater than 0.5% and there is persistent CAR T-cell expansion 3 months after T-cell infusion, we will perform clonality studies (an extra 5 ml of blood). Further evaluations may be done based on clinical decisions.
- Immunophenotyping
- Serum or plasma cytokines/chemokines levels

All of these tests may not be performed on every specimen as a limited number of cells will be available. In general, priority will be given to PCR assays to detect infusion of T-cell lines.

Serum from blood drawn for functional studies on week 0 and week 4 will be stored for measurement of human anti-mouse antibodies (HAMA) in the event of a suspected immunologic reaction.

The lesser of either 60ml or 3ml/kg of patient weight of peripheral blood will be drawn on any one day for these assays. If a patient's hemoglobin is less than 7.0 g/dl on a previous evaluation at any of the evaluation times, the amount of blood drawn for the evaluation will be reduced and may be obtained over more than one venipuncture.

Percutaneous biopsy of at least 1 tumor lesion is a required component of this study, and will be performed at weeks 2-4 post treatment by an interventional radiologist or a surgeon. The biopsy costs will be covered by the study. The specimen will be tested for the presence of CAR T cells and their potential effect on the microenvironment. If patient is unable to complete the biopsy, he/she will still be allowed to remain on study for post-treatment follow up (Section 7.1).

7.5 Safety Testing

7.5.1 RCR testing by PCR: RCR testing will be performed Week 0, months 3, 6, 12. If all post treatment samples are negative during the first year the subsequent samples will be archived yearly for a total of 15 years. Aliquot of cells and serums will also be archived for use in future studies for RCR as required by the.

7.5.2 The PI will designate the choice of assay depending on the mode of vector administration and the clinical application. If all post-treatment assays are negative during the first year, blood will be obtained yearly for archiving cells and serum as per current FDA recommendations for long-term follow up of gene therapy patients. If any post treatment samples are positive, further analysis of the RCR will be undertaken, in consultation with CBER.

7.5.3 All positive RCR results will be reported immediately to the FDA as an adverse experience in the form of an IND safety report.

7.5.4 The following will be obtained yearly for 15 years: Brief clinical history – with specific emphasis on clinical outcomes suggestive of retroviral disease such as cancer, neurologic disorders or other hematologic disorders.

7.5.5 For any patients who die or develop a second neoplasm, significant hematologic or neurologic disorder during the trial, a biopsy sample of the neoplastic tissue or pertinent autopsy tissue will be assayed for RCR (if a sample can be obtained).

7.5.6 Other Tissues: Patients will undergo tumor biopsy post-treatment while they are on study, and a sample of this will be used to assess the presence/absence of the retrovirally transduced cells (if a sample can be obtained).

7.5.7 Long term follow up for patients receiving gene transfer product will be conducted as per SOP J02.51.XX.

7.6 Determination of antitumor responses

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. Evaluations of tumor size will be performed within 4 weeks of beginning treatment and 4 weeks (range 4 – 6 weeks) after 15.GPC3-CAR T-cell infusion. In addition, we will determine AFP as a tumor marker in the peripheral blood of patients pre-chemotherapy, pre-infusion, at 1, 2, and 4 weeks post T-cell infusion in patients with AFP producing tumors.

Definitions
Response and progression will be evaluated in this study using the international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) Committee [82, 83] and by the Immune-related Response Criteria [84, 85].

RECIST criteria: Changes in only the largest diameter (unidimensional measurement) of the tumor lesions are used. Measurable lesions are defined as those that can be accurately measured in at least one dimension (unidirectional, longest diameter to be recorded) as >20 mm with conventional techniques (CT) or as >10 mm with spiral CT scan or MRI. The investigator will identify up to 10 measurable lesions to be followed for response. Serial measurements are to be done with CT or MRI. The same method of assessment is to be used to characterize each identified and reported lesion at baseline and during follow-up.

Complete Response (CR): Disappearance of all target lesions

Partial Response (PR): At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD.

For patients with metastatic disease, who have accessible tumor sites, we will request a biopsy to confirm the imaging results and analyze the tumor infiltrating T-cell populations. To determine the presence of 15.GPC3-CAR T cells, we will use Q-PCR for the transgene.

Immune-related Response Criteria (IrRC): Pseudoprogression corresponds to increase of lesion size in response to therapy, which has been observed particular for melanoma, brain tumors after receiving immunotherapy and HCC after administration of sorafenib a multi-tyrosine-kinase inhibitor [84-87]. Since AFP serum levels correlate with disease status in HCC [88, 89], we will use AFP levels to differentiate between progression and pseudoprogression in patients with AFP-secreting HCC. For patients with AFP-negative HCC additional diagnostic tests will be performed in close consultations with the patient's primary physician to differentiate between both possibilities. To fully describe the responses of patients treated on this protocol, we will report responses based on the recently described IrRC and all lesions at enrollment will be measured bidirectionally as target lesions:

Response by decreasing in AFP levels: Only applicable to patients with elevated AFP at the time of enrollment. AFP levels will be measured at the time of starting

lymphodepletion (Day -4) and at the end of safety assessment (Week 4).

Complete Response (CR): Disappearance of all lesions. Nodes must regress to < 10 mm short axis. No new lesions. Normalization of AFP levels.

Partial Response (PR): ≥50% decrease in tumor burden compared with baseline*. At least half log decrease in AFP values.

Progressive Disease (PD): ≥25% increase in tumor burden compared with baseline, nadir, or reset baseline*. New lesions added to the tumor burden. Greater than 10% increase in AFP levels.

Stable Disease (SD): Neither partial response nor progressive disease. Less than 10% increase and less than half log decrease of AFP levels.

**If an increase in tumor burden is observed at the first scheduled assessment, the baseline is reset to the value observed at the first assessment.*

Both RECIST and IrRC will be reported and IrRC will take priority for study related questions. AFP based responses will be reported separately.

8.0 Statistical Consideration

8.1 Clinical Trial Design

The Primary objective of this phase I clinical study is to determine the safety of escalating doses of 15.GPC3-CAR T cells in patients with GPC3-positive solid tumors after lymphodepleting chemotherapy. Secondary objectives are to determine the maximum tolerated dose (MTD) of 15.GPC3-CAR T cells and the anti-tumor effects in these patients as per the roadmaps in Appendix IV.

8.2 Dose Limiting Toxicity

DLT will be defined as any of the following that may, after consultation with the FDA, be considered possibly, probably, or definitely related to the study cellular products.

- Any Grade 5 event
- Non-hematologic dose-limiting toxicity is any Grade 3 or Grade 4 non-hematologic toxicity that fails to return to Grade 2 within 72 hours
- Grade 2 to 4 allergic reaction to CAR T cell infusion.
- Grade 4 hematologic toxicity that persists for 28 days or greater.
- Grade 3 and 4 expected reactions due to CRS and neurotoxicity are seen with the use of CAR-based immunotherapy. Grade 3 cytokine release syndrome (CRS) infusion reactions and neurologic toxicity will only be reported to the FDA if they fail to return to Grade 1 within 7 days. Grade 4 CRS and neurologic toxicities will be reported to the FDA in an expedited fashion.

8.3 The Dose Escalation Method and sample size

Dose escalation is guided by the Bayesian optimal interval (BOIN) design[90, 91] in order to determine the maximum tolerated dose (MTD) of 15.GPC3-CAR T cells. Dose limiting toxicity (DLT) is defined in Section 8.2. Each treated patient will be followed for 4 weeks post the 15.GPC3-CAR T cell infusion for the evaluation of DLT at their specified dose level. If no dose limiting toxicities are detected, a maximum of three

additional doses of 15.GPC3-CAR T cells may be given on the same dose level. Patients will need to meet treatment eligibility at the time of each start of lymphodepletion for reinfusions.

The BOIN design has algorithmic escalation/de-escalation rules like the traditional 3+3 design but allows for specification of various target toxicity rate, cohort size, and expanded accrual. With continued consistent toxicity monitoring, this design seeks to minimize incorrect escalation/de-escalation decision, thereby optimizing dose assignment for each patient. For a broad range of conditions, the BOIN design performs as well as the model-based continual reassessment method (CRM) designs in selecting the MTD, but has a lower risk of overdosing or under dosing[92].

In this trial, three patients will be initially enrolled to the lowest dose level. There will be a gap of 2 weeks between the first and second patient on each dose level. When these three patients are evaluable for DLT after their 4-week toxicity follow-up, we will assign the next cohort of three patients according to the decision rule of the BOIN design. The BOIN design is a type of 'interval design', in which dose choices are made according to pre-specified toxicity tolerance intervals. In general, with a target toxicity rate of 0.30 for this trial, we will escalate the dose if the observed toxicity rate at the current dose is <= 0.2365, or de-escalate the dose if the observed toxicity rate at the current dose is >=0.3585. If the observed toxicity rate is between 0.2365 and 0.3585, another cohort of patients will be treated at the current dose. For safety, no dose level will be skipped during dose escalation. Enrollment on the next dose level will wait until the completion of toxicity evaluation period following the 15.GPC3-CAR T cell infusion (4 weeks post infusion) for all treated patients enrolled at the current dose level. Dose escalation/de-escalation will continue until the recommendation is to assign the next cohort to a dose level that already has 6 patients treated. Dose escalation/de-escalation is executed according to the rules in the table below:

Dose escalation/de-escalation rule for the BOIN design

Number of patients treated at the current dose	3	4	5	6	7	8	9
Escalate if # of DLT ≤	0	0	1	1	1	1	2
Treat additional 3 patients on current dose if number of DLT =	1	1	NA	2	2	2	3
De-escalate if # of DLT≥*	2	2	2	3	3	3	4
Eliminate dose level and all higher dose from further use if number of DLT≥	3	3	4	4	5	5	5

**At the lowest dose level, if the recommendation is to de-escalate AND the dose level has not been eliminated from consideration, additional patients will be accrued at the same dose level; otherwise the trial will stop.*

"Eliminate" in the table means that we eliminate the current and any higher dose levels from the trial because they are overly toxic. Therefore, we will prevent treating any future patients at these toxic dose levels. If the current dose is DL1 and the rule indicates dose de-escalation, we will treat the next cohort at DL0. If the number of DLTs at DL0 reaches the elimination boundary, we will suspend the trial for safety and report to FDA. If FDA approves and further dose de-escalation is necessary, half log decreases will be used on DL-1 and on DL-2.If the current dose is the highest dose and the rule indicates dose escalation, we will treat additional patients at the highest dose until 6 patients are already treated at the current (i.e. highest) dose.

Once dose escalation is finished and all patients are evaluable for DLT, we will

determine the MTD based on isotonic regression as specified in Liu and Yuan[90] specifically, the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there is no DLT that determine a MTD, the maximum dose level will be declared as the MTD. In case the lowest dose level is determined to be too toxic and the elimination boundary is reached, no MTD will be defined for the trial.

Hence, upon completion of the dose escalation, we anticipate that 15 patients will be treated with a total 6 of patients accrued at the MTD level. Depending on observed toxicities, we expect that at most 24 patients will be infused in the phase-I trial.

The first three patients on this study will be treated at DL1.

DLTs will be evaluated from start of lymphodepleting chemotherapy to Day 28 (4 weeks) post-CAR T-cell infusion. We anticipate that we will need to procure no more than 26 patients to enroll 15-24 evaluable patients.

8.4. Stopping rules

The enrollment to the study will be held if DLT0 is overly toxic that the number of DLTs at this dose level reaches the elimination boundary. This enrollment hold rule does not mandate automatic closure of the study. It serves a rule to trigger the oversight by FDA. The FDA will review all available data on any SAE where the relationship to CTL is suspected. Based on the evaluations, the FDA may recommend modifications to the study in order to ensure subject safety or recommend stopping the trial.

8.5 Data Analysis

8.5.1. Safety Analysis of Adverse Event Data

All patients who received CAR T-cell infusions will be included in the safety analysis. All analyses will be performed separately for each dose level. Safety and toxicity outcomes will be summarized for the overall patient group and by dose levels. Adverse event data and corresponding toxicity grades four weeks after the T-cell infusion and during long-term follow-up will be summarized in the form of tables. Incidence tables will be generated to summarize incidence of patients reporting at least one episode of each specific adverse event, incidence of adverse events causing withdrawal and incidence of serious adverse events. The total number of episodes for each event reported (Frequency Table), the severity and attribution to study therapy of each episode reported (Severity Table and Attribution Table) will also be displayed.

Listings of adverse events by patients will include the time to onset, the duration of each event, the severity of each event, and the relationship of the event to study therapy, whether it was a serious event, and whether it caused withdrawal. Safety data will be summarized.

8.5.2. Clinical response data

Response rates will be estimated as the percent of patients whose best response is either complete response or partial response (see Section 7.6) by combining the data from lesions recorded via imaging or by AFP for each patient pre- and post-infusion. These data will be evaluated separately by both RECIST and IrRC. To compare with historical data, a 95% confidence interval will be calculated for the response rate.

The analysis of time to progression will include all patients who are considered evaluable for response. This will include patients that have had pre- and post-

therapy imaging and that are off-treatment but continue to be enrolled on the study protocol and that have been previously evaluated by RECIST and/or IrRc criteria. The probability of time to progression at 6 months will be summarized. Time to progression will be estimated using the product-limit method of Kaplan and Meier, and their associations with biologic biomarkers will be studied by the Cox regression in an exploratory way. Time to progression is defined as the number of days from enrollment to: (1) disease progression; (2) death because of treatment complications; or (3) last patient follow-up whichever is first. Patients will be considered to have experienced a progression event if (1) or (2) occurs. Otherwise, the patient will be considered censored for time to progression.

8.5.3. Analysis of Laboratory Data

Descriptive statistics (means, standard deviations, medians and ranges) at pre-infusion and at 1, 2, and 4 weeks post T-cell infusion will be calculated. Laboratory data collected at 3-month intervals for the first year will also be summarized. Scatter plots depicting laboratory values at each time point for each patient will also be generated. In order to analyze changes in laboratory values, a shift table with Stuart-Maxwell chi-square analysis of the change in the normal range from pre-infusion to post infusion time points (using high, normal, low) will be performed. When appropriate, these tables are collapsed and the McNemar's test applied in place of the Stuart-Maxwell test.

8.5.4. Analysis of Expansion and Persistence of T cells

Despite the small patient numbers, a data-dense study will be generated due to the repeat measurements on proliferation, immune function, etc. on each patient. Descriptive statistics using mean ± SD, medians and ranges will be calculated to summarize repeated measurements of T-cell expansion (increasing T-cell levels in the peripheral blood over time), and persistence (infused T cells continuing at detectable levels over time) following infusion as measured by PCR. Plots of growth curves will be generated to graphically illustrate patterns of survival and expansion of T cells as well as immune response. The validity of the normality assumption on these data will be tested and appropriate transformations will be considered whenever indicated.

To compare the persistence of 15.GPC3-CAR T cells among the four dose levels, we will calculate the area under the growth curves (AUC) over time for T-cell frequencies. After appropriate log-transformation, ANOVA and t-test will be used to compare the AUCs with Bonferroni adjustment for multiple comparisons. Alternative strategies such as repeated measurement models will be applied to compare immunological and virological efficacy across dose levels. We will use a type-I error of 10% in the analysis because of the small sample size.

The modeling strategies proposed here are amenable to these types of data but will however be considered exploratory and interpreted with caution due to limited study power and high type-I error. The results of this study will not be definitive but only exploratory.

9.0 Off Study Criteria

Patients are considered to be off study if they meet the following criteria:

9.1 Completion of study specified procedures

9.2 Refusal of further study follow up by patient or legal guardian

9.3 Lost to follow up

9.4 Death

Any questions regarding patients on this study should be addressed to Dr. Heczey at 832- 824-4233 Or Dr. Steffin at 832-824-4745.

10.0 Records to be kept

The criteria listed in the NCI Common Toxicity Criteria Scale will be used in grading toxicity. (Version 4.X located at http://ctep.cancer.gov) with the exception of CRS and neurological toxicities that are related to T-cell infusions. CRS and neurological toxicities will be graded according to Appendix VI.

The CAGT research nurse/coordinator will maintain a database documenting on study information, adverse events, off study notification and death information. The dates and doses of therapy as well as clinical chemistries, hematologic parameters, the clinical status and occurrence of any adverse events and subsequent interventions are to be kept on all patients.

Imaging reports
Biopsy and surgical summaries
Autopsy summaries, where appropriate
Informed consent documents

All required clinical evaluation records will be the responsibility of Dr. Heczey and Dr Steffin who will also be responsible for analysis of the clinical outcome and toxicity.

11.0 Reporting Requirements

11.1 Register all patients with Cell and Gene Therapy Research Coordinator.

11.2 Enter all patients by contacting Drs. Heczey or Steffin. The following data will be captured:

Eligibility
On study
Concomitant medication
Off study
Flow sheets
Adverse event
CRS Adverse event (if applicable)
Neurotoxicity Adverse event (if applicable)
Death

11.3. Drug Toxicity and/or Adverse Reactions

11.3.1 Adverse events will be collected as per SOP J 02.05.XX and SOP J 02.75.XX. Data on adverse experiences/toxicities regardless of seriousness, must be collected for documentation purposes only for 4 weeks after the study drug/biologic.

11.3.2 Serious adverse events will be collected as per SOP J 02.06.XX. Reporting of serious adverse events related to gene transfer will continue for 15 years per SOP J 02.51.XX.

11.3.3 Grade 3 cytokine release syndrome (CRS) infusion reactions and neurologic toxicity will be reported to the FDA if they fail to return to Grade 1 within 7 days. Grade 4 CRS and neurologic toxicities will be reported to the FDA in an expedited fashion.

12.0 Informed Consent

All patents and/or their legal guardian must sign a document of informed consent consistent with local institutional and Federal guidelines stating that they are aware of the investigational nature of this protocol and of the possible side effects of treatment, including tumor biopsy (Section 7.5). Further, patients must be informed that no efficacy of this therapy is guaranteed, and that unforeseen toxicities may occur. Patients have the right to withdraw from this protocol at any time. No patient will be accepted for treatment without such a document signed by him or his legal guardian. Full confidentiality of patients and patient records will be provided according to institutional guidelines

13.0 Clinical Trial Oversight and Monitoring

This protocol will be conducted in accordance with the Cell and Gene Therapy Monitoring Plan on file with the FDA.

This protocol will be monitored in accordance with the current Data Safety Monitoring Plan. of the Dan L Duncan Cancer Center at Baylor College of Medicine

The conduct of this clinical trial will be evaluated in accordance with the Texas Children's Cancer Center and Center for Cell and Gene Therapy Quality Assurance Policy and Procedure Plan.

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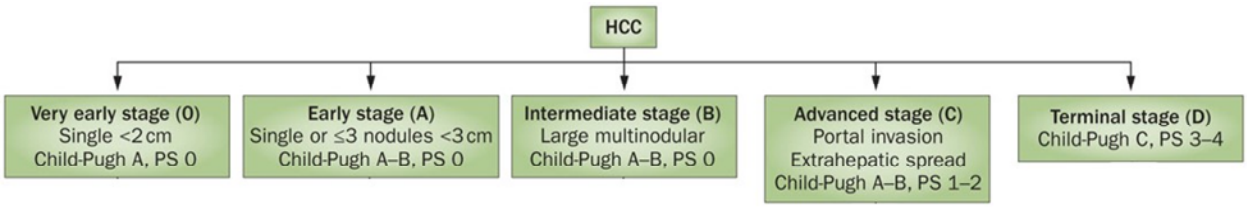
Appendix I – Karnofsky/Lansky Performance Score

Performance Status Criteria ***Karnofsky and Lansky performance scores are intended to be multiples of 10***			
Karnofsky (age ≥16)		Lansky (age<16)	
Score	Description	Score	Description
100	Normal, no complaints, no evidence of disease	100	Fully active, normal.
90	Able to carry on normal activity, minor signs or symptoms of disease	90	Minor restrictions in physically strenuous activity
80	Normal activity with effort; some signs or symptoms of disease.	80	Active, but tires more quickly
70	Cares for self, unable to carry on normal activity or do active work.	70	Both greater restriction of and less time spent in play activity
60	Required occasional assistance, but is able to care for most of his/her needs.	60	Up and around, but minimal active play; keeps busy with quieter activities.
50	Requires considerable assistance and frequent medical care.	50	Gets dressed, but lies around much of the day; no active play, able to participate in all quiet play and activities.
40	Disabled, requires special care and assistance.	40	Mostly in bed; participates in quiet activities
30	Severely disabled, hospitalization indicated. Death not imminent.	30	In bed; needs assistance even for quiet play.
20	Very sick, hospitalization indicated. Death not imminent.	20	Often sleeping; play entirely limited to very passive activities
10	Moribund, fatal processes progressing rapidly.	10	No play; does not get out of bed

Appendix II - Barcelona Clinic Liver Cancer Stage



The BCLC algorithm classifies HCC into five stages—based on the extent of disease, Child-Pugh score, and ECOG performance status—that enables prognostication and informs allocation of first-line treatment. If the proposed first-line treatment is contraindicated owing to a patient's clinical status, the treatment approach recommended for the subsequent disease stage should be considered. For example, a patient with early stage HCC (BCLC stage A) might benefit from TACE. Abbreviations: BCLC, Barcelona Clinic Liver Cancer (group); HCC, hepatocellular carcinoma; PS, performance status

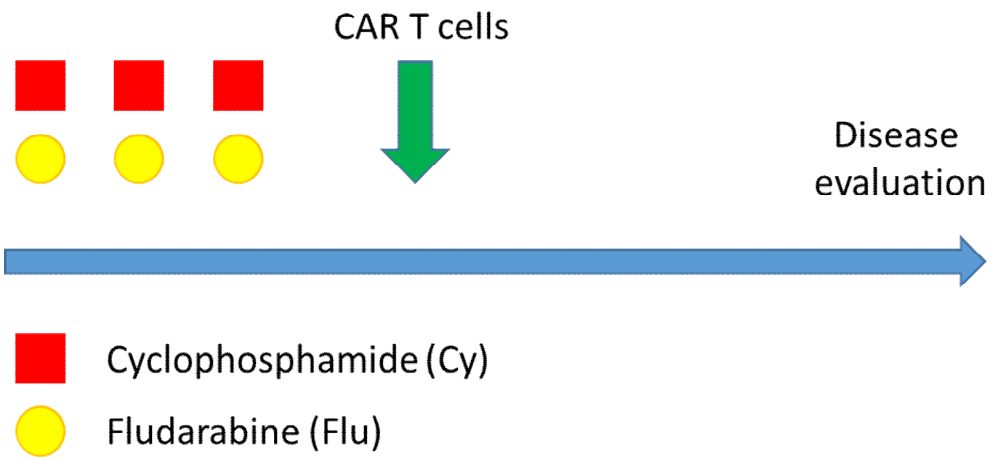
Appendix III - Child-Pugh-Turcotte Score

Measure	1 point	2 points	3 points	units
Bilirubin (total)	<2	2-3	>3	mg/dl
Serum albumin	>35	28-35	<28	g/L
INR	<1.7	1.71-2.20	>2.2	No unit
Ascites	None	Suppressed with medication	Refractory	No unit
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication	Grade III-IV (refractory)	No unit

Interpretation: The score employs five clinical measures of liver disease. Each measure is scored 1-3, with 3 indicating most severe derangement. Chronic liver disease is classified into Child-Pugh Turcotte class A (5-6), B (7-9), and C (10-15), employing the added score from above. Hepatic encephalopathy: Grade 1 - Trivial lack of awareness; euphoria or anxiety; shortened attention span; impaired performance of addition or subtraction. Grade 2 - Lethargy or apathy; minimal disorientation for time or place; subtle personality change; inappropriate behavior. Grade 3 - Somnolence to semistupor, but responsive to verbal stimuli; confusion; gross disorientation. Grade 4 – Coma.

Appendix IV – Roadmaps

Lymphodepletion with Cyclophosphamide and Fludarabine



DAY	SCHEDULED START DATE	DATE STARTED	FLUDARABINE (30MG/M2/DOSE)	CYCLOPHOSPHAMIDE (500MG/M2/DOSE)	CAR T-CELL INFUSION
-4			_____ mg	_____ mg	
-3			_____ mg	_____ mg	
-2			_____ mg	_____ mg	
-1					
0 to +2					**X**

Infusions should be given following hospital/pharmacy recommendations however at a minimum cyclophosphamide should be infused over 1 hour and fludarabine should be infused over 30 minutes. Mesna, IV hydration and anti-emetics should also be provided following local institutional guidelines. T cell infusion will be given 2 to 4 days post completion of chemotherapy. For follow up post T-cell infusion please refer to patient-specific follow up schedule as outlined in Section 7 of the complete protocol,

Appendix V -
Potential Side Effects of Lymphodepletion Chemotherapy

Risks and side effects related to <u>CYCLOPHOSPHAMIDE</u> include those which are:

Likely	Less likely	Rare but serious
• Loss of appetite • Nausea • Vomiting • Fewer white blood cells in the blood. • A low number of white blood cells may make it easier to get infections. • Hair loss • Decreased ability of the body to fight infection • Absence or decrease in the number of sperm which may be temporary or permanent which may decrease the ability to have children	• Abnormal hormone function which may lower the level of salt in the blood • Abdominal pain • Diarrhea • Fewer red blood cells and platelets in the blood • A low number of red blood cells may make you feel tired and weak. • A low number of platelets may cause you to bruise and bleed more easily. • Bleeding and inflammation of the urinary bladder • Absence or decrease monthly periods which may be temporary or permanent and which may decrease the ability to have children • Temporary blurred vision • Nasal stuffiness with IV infusions • Skin rash • Darkening of areas of the skin and finger nails • Slow healing of wounds • Infections	• Heart muscle damage which may occur with very high doses and which may be fatal. • Abnormal heart rhythms • Damage and scarring of lung tissue which may make you short of breath • A new cancer or leukemia resulting from this treatment. • Damage or scarring of urinary bladder tissue • Severe allergic reaction which can be life threatening with shortness of breath, low blood pressure, rapid heart rate chills and fever • Infertility which is the inability to have children

Risks and side effects related to FLUDARABINE (IV) include those which are:

Likely	Less likely	Rare but serious
• Loss of appetite • Nausea or the urge to vomit • Decreased number of red blood cells, white blood cells (neutrophil/granulocyte), and/or platelets (a blood cell that helps clot blood) • Muscle weakness of the whole body • Cough • Shortness of breath • Fatigue or tiredness • Fever • Infection • Pain • Increased risk of unusual infections lasting more than 6 months	• Skin rash with the presence of macules (flat discolored area) and papules (raised bumps) • Diarrhea • Irritation or sores in the lining of the mouth, voice box, throat, and windpipe • Vomiting • Peripheral Neuropathy - Commonly known as "pins and needles," where part of the body (typically a foot or hand) begins to tingle and becomes numb, or "falls asleep" • Blurred vision, double vision and/or loss of vision (blindness) • Fear of light • Inflammation of the lungs that may cause difficulty breathing and can be life-threatening • Chills • An increase in the number of a type of white blood cell (called eosinophils) in the blood • Agitation or restlessness • Confusion • Weakness or paralysis (loss of muscle function) caused by damage to peripheral nerves (those nerves outside of brain and spinal cord) • Inflammation (swelling and redness) or degeneration of the peripheral nerves (those nerves outside of brain and spinal cord) causing numbness, tingling, burning • Pain of the urinary tract • Inflammation (swelling and redness) of the paranasal sinuses which	• Severe rash with redness, pain and/or blisters. When pressure is applied to an area, the skin will detach from the lower layers. • A rare autoimmune disorder called Evan's syndrome in which the body makes antibodies that destroy the red blood cells, platelets and white blood cells • Sudden damage to the red blood cells (hemolytic anemia) which could cause a rapid decrease in the number of red blood cells such that you may be tired, weak, feel short of breath, and may require a blood transfusion • Coma and/or abnormal brain function • Convulsion or seizure • Blindness • A rare disorder that damages the material that covers and protects nerves in the white matter of the brain. The disorder may cause headaches, loss of coordination, clumsiness, loss of language ability, memory loss, vision problems, and weakness of the legs and arms that gets worse. • Inflammation (swelling and redness) of the bladder not due to urinary tract infection • Severe potentially life-threatening damage to the lungs which can lead to fluid in the lungs • Bleeding from the lungs • Kidney damage which may require dialysis

	may or may not be a result of infection	

Appendix VI -
Grading of CRS and Neurological Toxicities

CRS Grading Scale

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

Neurological Toxicity Grading Scale

Grade	Symptoms
1	• Somnolence-mild drowsiness or sleepiness • Confusion-mild disorientation • Encephalopathy-mild symptoms • Dysphasia-not impairing ability to communicate
2	• Somnolence-moderate, limiting instrumental ADL* • Confusion-moderate disorientation, limiting instrumental ADL* • Encephalopathy-limiting instrumental ADL* • Dysphasia-moderate impairing ability to communicate spontaneously
3	• Somnolence – obtundation or stupor • Confusion-severe disorientation, limiting self-care-ADL* • Encephalopathy-limiting self- care ADL* • Dysphasia-severe receptive or expressive characteristics, impairing ability to read, write or communicate intelligibly
4	• Life-threatening Consequences of Grade 3 Toxicities • Urgent Intervention Indicated • Mechanical Ventilation
5	• Death

*ADL: activities of daily living