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Official Title	A Cancer Research UK Phase I trial of anti-GD2 chimeric antigen receptor (CAR) transduced T-cells (1RG-CART) in patients with relapsed or refractory neuroblastoma.
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CANCER RESEARCH UK

Centre for Drug Development

A Cancer Research UK Phase I trial of anti-GD2 chimeric antigen receptor (CAR) transduced T-cells (1RG-CART) in patients with relapsed or refractory neuroblastoma.

Sponsor protocol number: CRUKD/15/001

EudraCT number: 2013-004554-17

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[redacted]

Sponsor: Cancer Research UK

Centre for Drug Development

[redacted]

PARTICIPATING INVESTIGATORS AND CENTRES:

Details of Principal Investigators and Investigational Sites are recorded on the Participating Investigators and Centres list in the Sponsor's Trial Master File.

VERSION HISTORY:

Version No.	Date of issue	Reason for update
1.0	15Apr2015	Version submitted to the MHRA for approval
2.0	06Jun2015	Version resubmitted to the MHRA after being revised to address their comments and submitted to GTAC.
3.0	23Dec2015	Substantial amendment to change the cryopreservation media for 1RG-CART and revise main trial exclusion criteria 1; plus other non-substantial changes.
4.0	08Dec2016	Substantial amendment to allow for single patient cohorts (where appropriate); revise the regimens used in dose escalation; revise the time window and criteria for patients receiving a second dose after a failed first dose; correct the definition of persistence of 1RG-CART in the blood; revise main trial exclusion criterion 4; amend the timing of the Day 18 visit; plus other non-substantial changes. Not approved by GTAC and MHRA.
5.0	31Jan2017	A revised version of the previous amendment incorporating all changes above but reinstating a 21 day observation period after the first patient in each dose level as requested by the MHRA.
6.0	24May2017	Non-substantial changes to: the pharmaceutical section to clarify that 1RG-CART may be stored in cryobags as well as vials; wording of Sponsor and Investigator signature pages updated.
7.0	25Apr2018	To expand the second dose criteria and to allow a second manufacture for patients in Dose Level 1 who have insufficient stored 1RG-CART for a second dose in the currently recruiting dose level; plus other non-substantial changes.
8.0	18Oct2019	Non-substantial changes: change of Sponsor address; [redacted] restructuring of Section 8 to align with objectives/endpoints; correction of starting point for measurement of overall survival and progression-free survival; [redacted] other minor clarifications/corrections.

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

	Abbreviation	Definition
	1RG-CART	Autologous T-cells transduced with the MP10413 retrovirus and expressing both RQR8 and GD2 CAR
A	ABPI	Association of the British Pharmaceutical Industry
	AE	adverse event
	ALP	alkaline phosphatase
	ALL	acute lymphoblastic leukaemia
	ALT	alanine aminotransferase
	aPTT	activated partial thromboplastin time
	ASCT	autologous haemopoietic stem cell rescue
	ATIMP	advanced therapy investigational medicinal product
B	BP	blood pressure
	B-NHL	B-cell non-Hodgkin's lymphoma
	BSA	body surface area
C	CAIX	carbonic anhydrase IX
	CAR	chimeric antigen receptor
	CCLG	Children's Cancer and Leukaemia Group
	CEA	carcinoembryonic antigen
	CI	Chief Investigator
	CLL	chronic lymphocytic leukaemia
	CNS	central nervous system
	COG	Childhood Oncology Group
	CR	complete response / remission
	CRA	Clinical Research Associate
	CRP	C-reactive protein
	CRS	cytokine release syndrome
	CRUK	Cancer Research UK
	CSM	Clinical Study Manager
	CSR	clinical study report
	CT	computerised tomography
	CTA	clinical trial authorisation
	CTCAE	Common Terminology Criteria for Adverse Events (Version 4.02)
	CTIMP	clinical trial of an investigational medicinal product
	CXR	chest x-ray
D	DLT	dose limiting toxicity
	DMSO	dimethyl sulfoxide
E	EBV-CTL	Epstein-Barr virus-specific cytotoxic T-cells
	eCRF	electronic case report form
	EDC	electronic data capture
	EDTA	ethylene diamine tetra-acetic acid
	EU	European Union
F	FACS	fluorescence-activated cell sorting
	Fc	fragment crystallisable
	FDG	fluorodeoxyglucose
G	GCP	Good Clinical Practice
	GD2	disialoganglioside
	GFR	glomerular filtration rate
	GM-CSF	granulocyte-macrophage colony stimulating factor
	GOSH	Great Ormond Street Hospital
H	HACARA	human anti-CAR antibody
	HAS	human albumin solution
	Hb	haemoglobin
	HCG	human chorionic gonadotropin

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

	Abbreviation	Definition
	HIV	human immunodeficiency virus
	HRA	Health Research Authority
	HTLV	human T-cell lymphotropic virus
	HVA	homovallinic acid
I	^{123}I	iodine-123
	IB	Investigator's Brochure
	ICD	informed consent document(s)
	ICH GCP	International Conference on Harmonisation of Good Clinical Practice
	IFN-γ	interferon-γ
	Ig	immunoglobulin
	IL	interleukin
	IMP	investigational medicinal product
	INRC	International Neuroblastoma Response Criteria
	INRG	International Neuroblastoma Risk Group
	irRC	Immune Related Response Criteria
	ITF	Investigator Trial File
	IV	intravenous
L	LDH	lactate dehydrogenase
M	mAb	monoclonal antibody
	MHC	major histocompatibility complex
	MIBG	metaiodobenzylguanidine
	MHRA	Medicines and Healthcare products Regulatory Agency
	MR	mixed response
	MRI	magnetic resonance imaging
	MRD	minimal residual disease
N	NCI	National Cancer Institute
	NE	not evaluable / inevaluable
	NHL	non-Hodgkin's lymphoma
	NIMP	non-investigational medicinal product
	nr	not reported
	NR	no response
P	PBMC	peripheral blood mononuclear cell
	PCR	polymerase chain reaction
	PD	progressive disease
	PET	positron emission tomography
	PI	Principal Investigator
	PR	partial response / remission
	PSRB	Protocol and Safety Review Board
	PT	prothrombin time
	PV	pharmacovigilance
Q	QP	Qualified Person
R	REC	Research Ethics Committee
	RECIST	Response Evaluation Criteria in Solid Tumours (Version 1.1)
	RP2R	recommended Phase II regimen
	RQR8	the short name given to Ritux-QBEND10-Ritux-CD8STK, a "sort-suicide" gene which when expressed on the T-cell surface allows (1) detection of RQR8 and (2) selective depletion of expressing cells when rituximab is administered to patients.
S	SAE	serious adverse event
	scFV	single-chain variable fragment
	SCT	stem cell transplant
	SD	stable disease

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

	Abbreviation	Definition
	SIADH	syndrome of inappropriate antidiuretic hormone secretion
	SIOPEN	International Society of Paediatric Oncology European Neuroblastoma
	SmPC	Summary of Product Characteristics
	SOP	standard operating procedure
	SPD	sum of the products of the two largest perpendicular diameters
	SPECT	single-photon emission computed tomography
	SUSAR	suspected unexpected serious adverse (drug) reaction
T	TCR	T-cell receptor
	TMF	Trial Master File
	TNF	tumour necrosis factor
	TSC	Trial Steering Committee
	TT	thrombin time
U	UCLH	University College London Hospitals
	UMT	User Management Tool
	USM	urgent safety measure
V	VGPR	very good partial remission
	VMA	vanillylmandelic acid
W	WBC	white blood cell
	WHO	World Health Organisation
	w/v	weight / volume percent

PROTOCOL SIGNATURES

Sponsor Signature:

The Sponsor has read and agrees to the protocol, as detailed in this document. I am aware of my responsibilities as the Sponsor under the UK Clinical Trials Regulations[1], the guidelines of Good Clinical Practice (GCP)[2], the Declaration of Helsinki[3], the applicable regulations of UK law and the trial protocol. The Sponsor agrees to conduct the trial according to these regulations and guidelines and to appropriately direct and assist sponsor's staff who will be involved in the trial, and ensure that all staff members are aware of their clinical trial responsibilities.

Name: _____

Title: _____

Signature: _____

Date: _____

1 The Medicines for Human Use (Clinical Trials) Regulations (S.I. 2004/1031) and any subsequent amendments to it.

2 ICH Harmonised Tripartite Guideline E6(R1): Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) Step 5, adopted by CPMP July 1996.

3 WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and all subsequent amendments including Oct 2013.

Investigator Signature:

I have read and agree to the protocol, as detailed in this document. I am aware of my responsibilities as an Investigator under the UK Clinical Trials Regulations[1], the guidelines of Good Clinical Practice (GCP)[2], the Declaration of Helsinki[3], the applicable regulations of the relevant NHS Trusts and the trial protocol. I agree to conduct the trial according to these regulations and guidelines and to appropriately direct and assist the staff under my control, who will be involved in the trial, and ensure that all staff members are aware of their clinical trial responsibilities.

Investigator's Name: _____

Name of site: _____

Signature: _____

Date: _____

1 The Medicines for Human Use (Clinical Trials) Regulations (S.I. 2004/1031) and any subsequent amendments to it.

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1 PROTOCOL SUMMARY

1.1 Full Title

A Cancer Research UK Phase I trial of anti-GD2 chimeric antigen receptor (CAR) transduced T-cells (1RG-CART) in patients with relapsed or refractory neuroblastoma.

1.1.1 Short Title

A Phase I trial of anti-GD2 T-cells (1RG-CART).

1.2 Clinical Trial Objectives and Endpoints

Primary objectives	Endpoints
To evaluate the feasibility of 1RG-CART therapy in patients with relapsed or refractory neuroblastoma.	The percentage of patients who commence T-cell processing (defined as enrolling for leukapheresis/venepuncture) and who are subsequently evaluable for 1RG-CART survival on or after Day 14.
To assess the safety and tolerability of 1RG-CART therapy in patients with relapsed or refractory neuroblastoma.	Determine the incidence, severity (grading according to National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE] Version 4.02 or Appendix 1 for cytokine release syndrome [CRS]) and causality of adverse events (AEs).
To determine the recommended Phase II regimen (RP2R) for 1RG-CART therapy in patients with relapsed or refractory neuroblastoma.	Determine a dosing regimen for Phase II evaluation based on safety and pharmacodynamic data.

The secondary and tertiary objectives / endpoints of the trial can be found in Section 3.1.

1.3 Design

This is a two centre, open label, first-in-human, dose escalation and expansion, Phase I trial in patients with relapsed or refractory neuroblastoma. A detailed description of the trial design can be found in Section 3.2. The dose escalation phase of the trial will consist of cohorts of at least one patient until evidence of 1RG-CART engraftment, 1RG-CART-related toxicity (other than minor non-specific side effects, e.g. Grade 1-2 infusion-related reactions), and/or anti-tumour efficacy is seen. In case of uncertainty, one or more additional patients may be enrolled in a cohort.

1.4 Administration Schedule

Each cohort of one or more patients will receive 1RG-CART given as a slow intravenous (IV) injection or infusion (with a maximal infusion rate of 20 mL/m^2/min) on Day 0.

The first cohort of patients will receive $1x10^7$ 1RG-CART/m^2 on Day 0 without any preparative lymphodepleting regimen (Dose Level 1).

The second cohort of patients will be given 300 mg/m^2/day of cyclophosphamide each day on Days -4 to -1 (total of four doses), followed by $1x10^7$ 1RG-CART/m^2 on Day 0 (Dose Level 2).

The third cohort of patients will be given 300 mg/m^2/day of cyclophosphamide each day on Days -7 to -4 (total of four doses) and 25 mg/m^2/day of fludarabine each day on Days -8 to -4 (total of five doses), followed by $1x10^7$ 1RG-CART/m^2 on Day 0 (Dose Level 3).

The fourth cohort of patients will be given the same conditioning regimen as for Dose Level 3, followed by $1x10^8$ 1RG-CART/m^2 on Day 0 (Dose Level 4).

If 1RG-CART survival is not considered optimal in earlier dose levels and improved efficacy seems likely with a higher 1RG-CART dose, a fifth dose level may be explored. Patients in this cohort will be given the same conditioning regimen as for Dose Levels 3 and 4, followed by $5\text{-}10x10^8$ 1RG-CART/m^2 on Day 0 (Dose Level 5).

In most patients, it is expected that 1RG-CART will only be given once. However, patients may receive a second dose of 1RG-CART as described in Section 5.4.1.

If adequate 1RG-CART survival and anti-tumour efficacy is achieved in a cohort, then subsequent dose levels may be omitted and the expansion phase of the trial may commence. Persistence of transduced T-cells (defined as $\geq 0.02x10^9$/L transduced T-cells in peripheral blood) for at least 2 weeks after infusion in the majority of patients in a cohort is considered an indicator of successful engraftment.

In the expansion phase, additional patients will be recruited to the most favourable cohort (to give a total of nine patients treated at this dose level) to further evaluate safety, 1RG-CART engraftment and survival, and anti-tumour efficacy.

See Section 5.1 for further details.

1.5 Treatment Group

Patients with relapsed or refractory neuroblastoma. It is expected that all patients will be in the paediatric age range, although there is no upper age limit for the trial.

1.6 Expected Accrural

It is expected that between 15 and 27 patients will be required to complete this trial, the final number depending on the number of patients evaluated at each dose level and the number of dose levels explored.

2 INTRODUCTION

2.1 Background

The investigational medicinal product (IMP), 1RG-CART, used in this trial is classed as an advanced therapy investigational medicinal product (ATIMP). The cyclophosphamide and fludarabine used for the conditioning regimen are also considered investigational medicinal products (IMPs). Rituximab is considered a non-investigational medicinal product (NIMP) in this trial as it will only be used as a rescue medication when necessary. Cyclophosphamide, fludarabine and rituximab (MabThera®) will all be used outside of their licensed indications. All patients will also receive a dose of antihistamine prior to administration of the 1RG-CART, and this will also be considered a NIMP. Generic drugs can be used and the dose and use of the antihistamine will be in line with its licensed indication.

2.1.1 Background on Neuroblastoma

Neuroblastoma is a rare embryonal tumour of the sympathetic nervous system, which occurs almost exclusively in children. It is the most common extracranial solid tumour in childhood and the most common malignant tumour in infants (i.e. children aged <1 year) [2, 3]. There are approximately 1,385 new cases per year in Europe [4], including approximately 100 cases per year in the UK [5]. Overall, 5-year survival for neuroblastoma has improved in recent decades, especially in infants. In Europe the 5-year overall survival between 1993-1997 was 66% compared with 37% between 1978-1982 [5].

Neuroblastoma is a very heterogeneous disease in terms of clinical presentation and prognosis. Important prognostic factors include age, stage, MYCN status, histopathological classification, and the presence or absence of segmental chromosomal aberrations. These clinical and biological variables have been combined into risk stratification systems. Approximately a third of patients fall into the high risk group, which is defined by the International Society of Paediatric Oncology European Neuroblastoma (SIOPEN) Group as including patients older than 1.5 years with Stage 4 tumours, and patients with MYCN-amplified tumours. Patients in the International Neuroblastoma Risk Group (INRG) high risk group have 5-year event-free survival rates of <50%, compared with >85% for patients in the very low risk group [6].

For patients with high risk neuroblastoma, standard first-line therapy consists of intensive chemotherapy (generally with platinum agents, etoposide, vincristine and cyclophosphamide), followed by myeloablative chemotherapy with autologous haematopoietic stem cell rescue (ASCT), radiotherapy to the primary tumour site and maintenance therapy with 13-cis retinoic acid (isotretinoin) and the monoclonal antibody (mAb), ch14.18 (within the context of a clinical trial for patients in the UK). Patients in the UK with relapsed or refractory high risk neuroblastoma are generally entered into clinical trials of salvage therapy where possible.

Survival after relapse is poor with only 20% of patients still alive after five years [7]. For patients with relapsed high risk disease, the prognosis is worse. For example, a retrospective review was conducted of patients entered into three consecutive national trials of salvage therapy in Germany from 1990–2007 [8]. Four hundred and fifty-one patients aged at least one year, who received ASCT during first-line treatment for high risk disease, were identified. Of these patients, 253 relapsed (56%) and 158 received salvage chemotherapy (35%), including 23 patients (5%) who underwent a second ASCT and 21 patients who underwent an allogeneic or haploidentical stem cell transplant (SCT). The 23 patients who underwent a second ASCT demonstrated a better median survival (2.08 years) and 3-year survival rate (43.5%) than the 74 patients who had no second chemotherapy (median survival 0.24 years, 3-year survival rate 4.0±2.6%) and the 135 patients who underwent second-line chemotherapy but did not undergo second ASCT (median survival of 0.89 years, 3-year survival rate 9.6%). However, by February 2010, only three of the 23 patients who had undergone a second ASCT were in complete remission (CR), three were in very good partial remission (VGPR), and one was in partial remission (PR). Fourteen of the 23 patients had died of disease and two had died of complications due to the second ASCT. These results illustrate the very poor prognosis of patients with relapsed high risk neuroblastoma and the need for effective new therapies for these patients.

2.1.1.1 Disialoganglioside Expression and Rationale for Targeting in Neuroblastoma

Disialoganglioside (GD2) is a sialic acid-containing glycosphingolipid expressed primarily on the cell surface. It is thought to play an important role in the attachment of tumour cells to extracellular matrix proteins [9]. GD2 is densely, homogenously and almost universally expressed on neuroblastomas [10, 11]. In normal tissues, GD2 expression is largely limited to skin melanocytes, and peripheral pain fibre myelin sheaths [12]. Within the central nervous system (CNS), GD2 appears to be an embryonic antigen [13] but is expressed at low levels on oligodendrocytes and within the posterior pituitary [12]. GD2 is also expressed on other cancers including medulloblastomas, melanomas, soft-tissue sarcomas, osteosarcomas, Ewing sarcomas and small-cell lung cancers [14]. Importantly, GD2 expression appears to persist after anti-GD2-directed antibody therapy in the great majority of patients with neuroblastoma [15, 16, 17].

The safety and efficacy of targeting GD2 has been demonstrated in trials involving the GD2 targeted antibodies, ch14.18 and 3F8 and their derivatives. The ch14.18 and 3F8 antibodies were found to produce clinical responses in patients with advanced refractory neuroblastoma in early Phase I and Phase II trials [18]. Ch14.18 also improved outcomes in a randomised Phase III trial in children with high-risk neuroblastoma who had achieved radiological remission after initial therapy [19]. When given in combination with granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin (IL)-2, concurrently with standard isotretinoin therapy after induction therapy and ASCT, ch14.18 significantly improved event-free survival (66±5% vs. 46±5% at 2 years, P=0.01) and overall survival (86±4% vs. 75±5% at 2 years, P=0.02 without adjustment for interim analyses). Randomisation within the trial was stopped early on the basis of positive interim results, and ch14.18 was offered to participants in the control arm who were progression-free [20]. The trial continues to recruit (according to National Institutes of Health Clinical trials database April 2014, trial identifier NCT01704716) but now all patients receive ch14.18. A form of ch14.18, manufactured from SP2/0 cells, was licensed in the United States and the European Union (EU) for the treatment of neuroblastoma (generic name dinutuximab, Brand name Unituxin™) in 2015.

2.2 Chimeric Antigen Receptor T-cell Therapy

The principle underlying CAR T-cell therapy is the generation of large numbers of autologous peripheral T-cells, which recognise a surface antigen expressed on malignant cells. This is achieved by ex vivo transduction of the patient's peripheral blood T-cells with a gene encoding a CAR. CARs are generated by grafting the antigen-binding region of a mAb to intracellular T-cell signalling domains. Introduction of genes encoding CARs into T-cells enables the T-cells to recognise and respond to antigen-bearing cells, without major histocompatibility complex (MHC) restriction. On binding to the target antigen, the T-cells become activated and cause tumour destruction via a number of mechanisms, including the release of perforins, granzymes, cytokines (such as interferon-gamma [IFN-γ] and tumour necrosis factor alpha [TNF-α]), T-cell surface ligands (such as FAS ligand and TNF-related apoptosis-inducing ligand [TRAIL]) and by activating other components of the immune system.

CAR T-cell therapy has been most extensively evaluated in B-cell malignancies, and encouraging results have been reported for patients with chronic lymphocytic leukaemia (CLL) and acute lymphoblastic leukaemia (ALL) using CD19-directed CAR T-cells [21, 22, 23, 24, 25]. Two trials of CAR T-cell therapy have been conducted in patients with neuroblastoma, including one trial in which the CAR was directed against GD2, as described in the next paragraphs.

In 2006, a neuroblastoma Phase I trial was reported in which T-cells were targeted against CD171, a cell surface adhesion molecule [26]. In this trial, expression of the CAR was achieved by electroporation of a coding plasmid. Stable expressing clones were expanded to enable doses ranging from $10^8/m^2$ up to $10^9/m^2$ and six patients were treated. Transduced T-cells were detected by polymerase chain reaction (PCR) in peripheral blood for up to 56 days after infusion. No treatment-related toxicity or anti-tumour efficacy was noted in this trial.

In a second neuroblastoma clinical trial, the CAR was directed against GD2 and was introduced to autologous T-cells by transduction using a retroviral vector [27, 28]. In this trial, the CAR was expressed in activated T-cells and also in Epstein-Barr virus-specific cytotoxic T-cells (EBV-CTL). The CAR-encoding vector was identical in each case except for a non-coding sequence, allowing researchers to determine whether CAR-expressing T-cells identified in vivo arose from activated T-cells or EBV-CTL. Transduced

T-cells from both sources were mixed in equal proportions and administered IV at total doses of 2 to $20x10^7/m^2$. Nineteen patients were treated (with 44 infusions) without any severe or dose limiting toxicities (DLTs). CAR-engrafted EBV-CTLs reached higher levels in vivo than CAR-engrafted activated T-cells but at 6 weeks both populations were low or undetectable. However, low-level persistence was detected beyond 6 weeks (for up to 96 weeks for CAR-expressing EBV-CTLs and for up to 192 weeks for CAR-expressing activated T-cells). Three of 11 patients with active disease at the time of the GD2 CAR T-cell infusion achieved a CR and two of these CRs were durable out to >21 and >60 months respectively. All three patients had persistent CAR T-cells at 6 weeks.

For further details on these and other CAR T-cell trials, please see the 1RG-CART Investigator's Brochure (IB).

2.3 1RG-CART Therapy

A major limitation of the first generation CARs used in the previous trials in patients with neuroblastoma was their failure to trigger proliferation or cytokine secretion in response to recognition of tumour target antigen. Physiological T-cell activation requires both engagement of the T-cell receptor (TCR) with its antigen as well as a co-stimulatory signal. However, tumour cells rarely express co-stimulatory molecules. Combining intracellular components of co-stimulatory molecules within the CAR construct circumvents this problem. Engagement of such second-generation CARs with their antigen-expressing targets results not only in lysis of the tumour cell but also T-cell proliferation in vitro. Typically, CD28 or 4-1BB have been used as the co-stimulatory endodomain. Simultaneous administration of CD19-CAR T-cells containing either a CD3ζ (CD3-zeta) or a fused CD28-CD3ζ endodomain to patients with CD19-expressing lymphoma, resulted in preferential expansion and persistence of CAR T-cells containing CD28-CD3ζ [29]. Third generation CARs have also been described which contain tri-partite endodomains including both a TNF family receptor endodomain (OX40 or 41BB), as well as CD28.

In this trial, transduction of autologous T-cells will be achieved using the retrovirus, [redacted] (referred to as MP10413 retrovirus hereafter). This retrovirus encodes two different transgenes:

- The CAR itself, [redacted] (referred to hereafter as GD2 CAR). [redacted]
- A sort-suicide gene, which is called RQR8. [redacted]

[redacted]

[redacted]

A

For further details on 1RG-CART, the MP10413 retrovirus, the CAR construct, and RQR8, please refer to the 1RG-CART IB.

2.3.1 Lymphodepletion as a Preparative Regimen for 1RG-CART Therapy

Preclinical and clinical trials have demonstrated that the function of infused T-cells can be enhanced by prior depletion of the patient's other lymphocytes. Lymphodepletion is hypothesised to create space for

infused T-cells to expand, to promote availability of essential cytokines, and to deplete regulatory T-cells. The cyclophosphamide and fludarabine lymphodepletion regimen planned for this clinical trial uses similar doses to those used as part of a minimal intensity conditioning regimen for stem cell transplantation in children with immunodeficiencies. The regimen was well tolerated in this group of children despite their high vulnerability to treatment-related morbidity due to their underlying condition [30]. The combination of cyclophosphamide and fludarabine has also been used to condition paediatric neuroblastoma patients prior to reduced-intensity allogeneic SCT [31, 32]. The same two lymphodepleting cytotoxic agents were also used in the Cancer Research UK (CRUK) sponsored trial of MFEz T-cells (Protocol No. PH1/105, EudraCT No. 2005-004085-16, [redacted]) and in several other trials in patients receiving CAR T-cell and other adoptive T-cell therapies. For further details on the lymphodepleting agents, please refer to the Cyclophosphamide and Fludarabine (1RG-CART Conditioning Regimen) IB.

2.3.2 Safety of 1RG-CART Therapy

2.3.2.1 Toxicities Associated with GD2-targeted Antibodies and their Derivatives

Although the mechanism of action of GD2 CAR T-cell therapy and mAbs targeting GD2 are different, some similarities in the safety profile would be expected, notably those specifically associated with GD2-targeting. Table 1 summarises common Grade 3-4 toxicities shared by ch14.18, 3F8, and the IL-2-conjugated antibody derivative, hu14.18-IL-2 (from Parsons et al., 2013 [18]). Pain is the most common toxicity associated with these agents and this appears to be increased by concomitant IL-2 but controlled by opioid premedication and concurrent opioid infusion. The pain is typically abdominal and/or pelvic in distribution. Hypersensitivity and fever are other common toxicities associated with anti-GD2-targeted antibody infusions (also increased by concomitant IL-2). Other notable toxicities include urticaria, hypertension, nausea, vomiting, diarrhoea, transient visual changes (mydriasis and accommodation defects), hypokalaemia, hyponatraemia and elevated liver transaminase levels. Decreases in platelets, leukocytes, neutrophils and haemoglobin have also been reported with hu14.18-IL-2 immunocytokine, and are likely to be due to the IL-2 component. The toxicities associated with these antibodies/derivatives (including pain) typically resolve within a few hours of completion of the infusion and 3F8 is commonly administered as an outpatient treatment. Infusion-related pain, in particular, may be related to complement activation or other mechanisms. Importantly, pain was not observed in the previous GD2 CAR T-cell trial by Pule et al. (2008) [27]. Accordingly, infusion-related pain would not be anticipated to occur with 1RG-CART infusions.

Some toxicities described for GD2-directed mAbs are typical of mAb infusions in general, and are seen (especially with the first infusion of mAbs) regardless of the antigen targeted (e.g. rituximab, alemtuzumab, trastuzumab). Therefore, such toxicities may not occur with 1RG-CART therapy, although the infusion of autologous cells is also associated with infusion reactions.

Table 1. Incidence of Grade 3-4 Toxicities Seen with 3F8, ch14.18 and hu14-18-IL-2 (from Parsons et al., 2013 [18])

Toxicity	3F8 + GM-CSF + isotretinoin [1] (n=85)	Ch14.18 + GM-CSF + IL-2 + isotretinoin [2] (n=299)	Hu14.18-IL-2 [3] (n=38)
Pain	1%	52%	31%
Urticaria	0	13%	5%
Rash	nr	nr	5%
Hypertension	2%	nr	0
Hypotension	0	18%	16%
Fever	5%	39%	40%
Capillary leak/oedema	0	23%	32%
Anaphylaxis/respiratory distress	3-4%	13-25%	11%
Serum sickness	0	1%	0
Nausea/vomiting	2%	3%	0
Diarrhoea	4%	13%	0
Abnormal liver enzymes	4-8%	10-23%	23%
Neutropenia	nr	nr	34%
Hyperbilirubinaemia	nr	nr	21%
Thrombocytopenia	nr	nr	42%
Anaemia	nr	nr	24%
Hypokalaemia	11%	35%	10.5%
Hyponatraemia	0	23%	5%

[1] Cheung et al., 2012 [33]
[2] Yu et al., 2010 [19]
[3] Shusterman et al., 2010 [34]
Abbreviations: nr = not reported, GM-CSF = granulocyte-macrophage colony stimulating factor, IL-2 = interleukin-2

2.3.2.2 Toxicities Associated with Genetically Modified CAR T-cells

Previously identified safety concerns with genetically modified T-cells include the following (reviewed in Maher, 2012 [35] and Heslop, 2010 [36]):

- ***Immediate infusion-related events:*** Fever and chills have been commonly described in patients with haematological malignancies receiving CD19 or CD20-directed CAR T-cells [35, 57]. In addition, the rapid onset of severe pulmonary symptoms has also been described. A patient with metastatic colon cancer received $1x10^{10}$ T-cells modified with a third generation CAR targeting HER2 and containing 4-1BB and CD28 co-stimulatory moieties, after intensive lymphodepletion [37]. The patient developed pulmonary toxicity within 15 minutes of the T-cell infusion, followed by cardiac arrest and subsequently died 4 days later. The events were associated with very high cytokine levels and may have been due to targeting of low levels of HER2 on pulmonary endothelium by very high numbers of transgenic T-cells leading to local cytokine hypersecretion and pulmonary leukostasis (i.e. an “on-target but off-tumour” effect – see below). Anaphylaxis has also been reported in a patient receiving CAR T-cells directed against mesothelin (see fourth bullet point below).

- ***Toxicities due to immune activation and cytokine storm:*** Immune activation and cytokine storms have been described in adult and paediatric patients with haematological malignancies treated with CD19-and CD20-targeted T-cell therapy [25, 38, 39, 57]. As would be expected from the mechanism of action of CAR T-cells, at least some level of cytokine release appears to be necessary for CAR T-cells to have efficacy, and the severity of cytokine release correlates with tumour bulk as well as anti-tumour activity. In one child who experienced a severe cytokine storm, cytokine blockade with etanercept and tocilizumab (in addition to glucocorticoids) was effective in reversing the syndrome and did not appear to inhibit the expansion or anti-leukaemic efficacy of the transduced T-cells [23]. Glucocorticoids were also used to treat CRS caused by anti-CD20 CAR

T-cells without eliminating the CAR T-cells [57]. However, in adult patients with sCSR, glucocorticoids (but not tocilizumab) also eliminated the anti-CD19 CAR T-cells and this appeared to lead to loss of anti-leukaemic efficacy. Of interest, CRS has been reported in a patient who received genetically unmodified T-cells with native receptors directed against tumour antigens [58]. The patient, who had bulky refractory Epstein-Barr virus (EBV)-associated lymphoma, developed the syndrome two weeks after receiving donor-derived, ex vivo expanded T-cells directed against EBV antigens through their native receptors. Cytokine release syndrome has also been reported after natural and bispecific antibodies and derivatives, especially those targeting CD3 (OKT3), CD52 (alemtuzumab) and CD20 (rituximab), and the CD28 super-agonist, TGN1412 (reviewed in Lee et al, 2014 [56]). Guidelines have been devised for monitoring patients receiving CAR T-cell therapy and for diagnosing and managing severe CRS in these patients (see Appendix 1 for grading of CRS according to Lee et al. (2014) [56] and Appendix 2 for indicators and management of severe CRS derived from Davila et al. (2014) [38].

- ***"On-target but off-tumour" effects:*** When allogeneic T-cells specific for minor histocompatibility antigens were given to treat leukaemic relapse, severe toxicity resulted from the unexpected presence of the target antigens on lung tissue [40]. Similarly, T-cells genetically modified with MART-1-specific T-cell receptors (which recognise a melanoma associated antigen) were associated with toxicities related to melanin-expressing cells in the inner ear and the retina (causing uveitis and hearing loss) [41]. T-cells directed against a MAGE-A3 epitope (another melanoma associated antigen) have also cross reacted with a related cardiac muscle protein resulting in fatal cardiac toxicity [42] and fatal neurotoxicity [68]. A CAR targeting carbonic anhydrase IX (CAIX) expressed by renal carcinoma cells produced liver toxicity because the target antigen was also expressed on bile duct epithelium [43]. This toxicity could be prevented by prior administration of a mAb to CAIX. CD19 is a specific antigen of B-cell lineage, which is expressed on the surface of malignant B-cells as well as normal B-lymphocytes. Therefore, B-cell aplasia is one of the complications of CD19 CAR T-cell therapy [69]. Finally, CAR T-cell therapy targeting carcinoembryonic antigen (CEA) caused severe dose-limiting colitis in patients with colorectal cancer [44].

- ***Toxicities due to immune responses against the CAR or vector-associated proteins:*** In a trial of CAR T-cells targeting CAIX in renal cell carcinoma, the majority of patients developed humoral and/or cellular immune responses to the CAR and/or viral vector [45]. Although this did not result in any apparent toxicity, it did appear to limit the duration of transduced T-cell survival in the circulation. Of note, patients in this study were not lymphodepleted prior to CAR T-cell infusion and they received multiple infusions, which may have increased the immunogenicity of the CAR T-cell treatment. Anaphylaxis has been described in a patient receiving a third dose of CAR T-cells directed against mesothelin [46, 47]. The patient experienced a cardiac arrest but was resuscitated and made a complete recovery. This event was thought to be due to IgE antibodies to the CAR itself, which included a scFv component derived originally from a murine antibody. The authors felt that the schedule of infusions was critical in this patient (3 infusions given over 49 days) because it allowed Ig switching (to IgE) to occur. As a result of this case, they modified their schedule for future patients so that the gap between infusions was no more than 10 days and all infusions had to be given within 21 days. Of note, the scFv targeting GD2 in the CAR expressed in 1RG-CART is fully humanised, and all other components of the receptor are human. These features are intended to minimise the risk of cross reactivity and possible host rejection and will also reduce the chance of anaphylaxis. The CAR expressed by 1RG-CART targets a different antigen (GD2), and 1RG-CART are intended to persist in the circulation and expand. In contrast, the CAR T-cells given to the patient who experienced anaphylaxis target the antigen mesothelin and the CAR expression is intended to be transient, lasting a few days only. These differences may also be important in the relative immunogenicity of the two CAR T-cell therapies. Importantly, all infusions of 1RG-CART will take place in a hospital environment with immediate facilities available to manage infusion-related reactions including anaphylaxis.

- ***Normal tissue 'bystander' damage:*** Damage to normal tissue surrounding tumour sites has been described. These effects are probably due to inflammation and/or necrosis of the normal tissue following CAR T-cell mediated tumour lysis. Symptoms/signs include GI haemorrhage in patients with lymphoma involving the GI tract, serous effusion and pulmonary consolidation with dyspnoea and respiratory distress in patients with lung parenchymal involvement with lymphoma [57].

2.3.2.3 Expected Safety Profile for 1RG-CART Therapy

Based on clinical experience with GD2-targeted mAbs, previous GD2 CAR T-cell therapy and other CAR T-cell therapies, potential toxicities of 1RG-CART therapy in patients with neuroblastoma include the following:

- Side effects of lymphodepleting chemotherapy (if given), notably infections related to neutropenia and/or lymphopenia. These are considered readily manageable in the context of advanced, relapsed neuroblastoma and the planned in-patient setting.
- Acute (occurring on the day of administration) non-specific or specific reactions to T-cell infusions, which could include allergic-type reactions to the T-cells, traces of culture-related products or the transgene, and/or exposure to dimethyl sulfoxide (DMSO) or human albumin solution (HAS). Anaphylaxis could potentially occur in patients given a second dose of 1RG-CART. Respiratory distress syndrome due to pulmonary leukostasis is also possible (although considered unlikely since patients will only be given low doses of T-cells and GD2 is not expressed in pulmonary endothelium). Symptoms could include rash, fever, rigors, bronchospasm, and hypotension. Infusion-related reactions are considered readily manageable in the context of advanced, relapsed neuroblastoma and the planned in-patient setting. All patients will receive supportive care including pre-medication with an antihistamine (see Section 5.1.3). Should acute infusion-related neuropathic pain occur (as seen with GD2-targeted mAb infusions), patients will be treated with analgesia regimens known to be effective in managing ch14.18-related pain (see Appendix 9).
- Toxicity from intense immune activation, including cytokine storm and/or macrophage activation syndrome (overlapping syndromes – see Rosado & Kim, 2013 [48]). This is considered the most important and most likely toxicity of 1RG-CART therapy in patients with neuroblastoma. Apart from general supportive care measures (including fluid administration, oxygen, ventilatory support, antiepileptics and inotropes if needed), potential treatments include cytokine antagonists, such as tocilizumab or a TNF antagonist (e.g. etanercept) or corticosteroids. Administration of rituximab (to activate the suicide gene product, RQR8, and trigger apoptosis of 1RG-CART) is considered unlikely to be necessary and could potentially exacerbate symptoms due to rituximab infusion-related cytokine release. However, rituximab administration would be considered if other methods failed to control the symptoms.
- Direct effects of CAR activity, notably neuropathic pain and/or peripheral neuropathy (due to GD2 expression on myelin sheathes), tumour lysis syndrome (generally extremely rare in neuroblastoma), and/or local inflammation at sites of disease. GD2 expression in the CNS and/or posterior pituitary could potentially lead to adverse CNS effects, including neurological disorders of the eyes (such as blurred vision, photophobia, mydriasis, fixed or unequal pupils, optic nerve disorder, eyelid ptosis and papilledema) or syndrome of inappropriate antidiuretic hormone secretion (SIADH). GD2 expression in melanocytes could potentially lead to pigment disorders, such as vitiligo, or increased susceptibility to sunburn. Rituximab (to activate the suicide gene) may be indicated for severe or progressive neuropathic pain that cannot be controlled with conventional analgesics (see Appendix 9) or for CNS effects, unless they are mild or transient. Patients with bulky disease at anatomic locations which could be hazardous, if acutely inflamed (notably where it might threaten an airway), will be excluded from the trial. Photophobia may be managed with supportive measures such as dimming the lights or the closing curtains. Patients/parents will be advised to take appropriate precautionary measures regarding sun exposure.
- Immunogenicity such as human anti-CAR antibody formation (HACARA) or formation of antibodies to RQR8 or other components of the transgene or vector. Given the unique expression of RQR8 and the GD2 CAR in transduced T-cells, antibody- or cell-mediated immunity against these products is likely to result in elimination of transduced T-cells (and therefore loss of efficacy) rather than unwanted immunological effects.
- Effects of integrating vectors, including insertional mutagenesis-driven T-cell lymphoproliferation and germ-line transmission of vector. Uncontrolled lymphoproliferation due to continued antigenic stimulation of 1RG-CART by GD2 expressed on normal cells after elimination of neuroblastoma could also occur. However, these toxicities are purely theoretical, and have never been observed in an engineered T-cell trial. First line treatment would be rituximab to activate the suicide gene.

The timing and management of potential toxicities of 1RG-CART therapy are illustrated schematically in Appendix 11 (note that timings should be regarded as uncertain and approximate). Section 3.5 provides detailed information on the potential use of rituximab in this trial (for further details on rituximab please refer to the Rituximab (1RG-CART Rescue Therapy) IB.

2.4 Rationale for the Proposed Trial

The purpose of this trial is to explore the feasibility of deploying autologous anti-GD2 CAR T-cells for the immunotherapy of neuroblastoma. The CAR T-cell trials employing second generation receptors and lymphodepleting conditioning regimes have produced objective clinical responses in patients with relapsed leukaemias. The current trial aims to evaluate similar CAR T-cells but directed against the antigen GD2. Neuroblastoma is well suited to this form of targeted therapy because of the homogeneous and almost universal expression of GD2 on the surface of neuroblastoma cells, and because of the poor prognosis of eligible patients. A successful outcome in this trial could pave the way for the development of the GD2 CAR T-cell therapy for additional GD2-positive malignancies.

1RG-CART will be administered by IV injection/infusion as is standard for T-cell adoptive transfer trials. As the CAR T-cells are designed to survive and proliferate on encountering antigen, no direct relationship is anticipated between cell dose and either efficacy or toxicity. Rather, clinical benefit is more likely to be observed in those patients in whom in vivo expansion successfully occurs. A likely key determinant of expansion will be prior lymphodepletion of the patients. For this reason this trial is designed to initially evaluate a phased introduction of lymphodepletion in successive patient cohorts. Only if there is insufficient expansion of T-cells following full lymphodepletion will the T-cell dose be escalated. Initially, a very modest dose of T-cells is planned to reduce the possibility of immediate adverse reactions, such as pulmonary leukostasis or severe cytokine storms induced by infused cells. The T-cell doses proposed for this clinical trial are based on those of the Phase I GD2 CAR T-cell trial performed in a similar patient group in the Texas Children's Hospital [27] and previous adoptive transfer T-cell trials in Epstein Barr Virus-positive malignancies [49, 50].

2.5 Selection of the Phase I Starting Dose and Schedule

1RG-CART will be administered to patients as a slow IV injection or infusion (with a maximal infusion rate of 20 mL/m^2/min) on Day 0. The starting dose (and the dose for Dose Levels 2 and 3) will be $1x10^7$ 1RG-CART/m^2, based on prior clinical experience with CAR T-cell therapy (pre-clinical studies have not been conducted since no suitable animal model exists). This is at the lower end of the dose of CAR T-cells infused in previous CAR T-cell trials, in which a second generation CAR construct was used (Table 2). The starting dose of CAR T-cells in this CRUK trial is also lower than that given in the first anti-GD2 CAR T-cell trial, in which a first generation CAR was used. The dose in that trial was 2 to $20x10^7$ cells/m^2 [27].

In this trial, it is expected that most patients will only receive a single dose of 1RG-CART. However, patients may receive a second dose of 1RG-CART as described in Section 5.4.1. Most CAR T-cell trials have involved administration of more than one dose of CAR T-cells. As many as 10 doses have been given to individual patients with no reports of adverse consequences associated with repeat dosing, other than anaphylaxis in one patient (see Section 2.3.2.2 for details of this case and the 1RG-CART IB for details of other CAR T-cell trials).

Table 2 Dose of Relevant Second Generation CAR T-cells (CD28-CD3ζ construct) Used in Published CAR T-cell Clinical Trials

Target	Patients[d]	Doses	Dose range	Disease	Reference
CD19	1	2	[a]$4x10^8$ [b]$1.4x10^8$ cells/m^2	B-NHL	[51]
CD19	8	2	0.3 to $3x10^7$ cells/kg [b]0.75 to $7.5x10^8$ cells/m^2	B-NHL	[52]
CD19	10	1	1 – $10x10^6$/kg (allogeneic T-cells)	B-cell malignancies	[61]
CD19	6	1-2	0.2-$8x10^8$ cells/m^2 per dose	Non-Hodgkin's lymphoma	[29]
CD19	9	1 (split in some cases)	[a]$1.8x10^8$ to $2.5x10^9$ [b]$6.4x10^7$ to $8.9x10^8$ cells/m^2	CLL/ALL	[24]
CD19	5	1	$1.5x10^6$ to $3x10^6$ cells/kg [b]$5.6x10^5$ to $1.1x10^6$ cells/m^2	ALL	[25]
CD19	16	1	$3x10^6$/kg	B-ALL	[38]
CD19	15	1	$1x10^6$ CAR-positive T/kg[c]	B-cell malignancies	[62]
CD19	21	1	$1x10^6$ to $3x10^6$/kg	ALL & NHL	[63]
CD19	7	1	$3x10^6$ to $3x10^7$/kg	CLL	[64]
CD19	6	1	0.2 to $2x10^8$ cells/m^2	B-cell malignancies	[65]
PSMA	7	1	1 to $3x10^7$/kg	Prostate cancer	[66]
LeY antigen	4	1	1.5 to $9.2x10^6$/kg	AML	[67]

[a] When whole body dose given a body weight of 70 kg was assumed to convert dose to m^2.
[b] Cell dose in mg/kg converted to dose in mg/m^2 using an allometric scaling factor of 25 based on a child bodyweight of 20kg.
[c] Dose reduced from 5x10 CART/kg during the study due to toxicity.
[d] Some patients are included in more than one publication (see 1RG-CART IB for more details).

Abbreviations: ALL=acute lymphoblastic leukaemia; AML=acute myeloid leukaemia; B-NHL=B-cell non-Hodgkin's lymphoma; CLL=chronic lymphocytic leukaemia; PSMA = prostate specific membrane antigen.

The rationale for the starting doses of cyclophosphamide and fludarabine can be found in the Cyclophosphamide and Fludarabine (1RG-CART Conditioning Regimen) IB.

3 TRIAL DESIGN

3.1 Clinical Trial Objectives and Endpoints

3.1.1 Primary Objectives and Endpoints

Primary objectives	Endpoints
To evaluate the feasibility of 1RG-CART therapy in patients with relapsed or refractory neuroblastoma.	The percentage of patients who commence T-cell processing (defined as enrolling for leukapheresis/venepuncture) and who are subsequently evaluable for 1RG-CART survival on or after Day 14.
To assess the safety and tolerability of 1RG-CART therapy in patients with relapsed or refractory neuroblastoma.	Determine the incidence, severity (grading according to NCI CTCAE Version 4.02 or Appendix 1 for CRS) and causality of adverse events (AEs).
To determine the recommended Phase II regimen (RP2R) for 1RG-CART therapy in patients with relapsed or refractory neuroblastoma.	Determine a dosing regimen for Phase II evaluation based on safety and pharmacodynamic data.

3.1.2 Secondary Objectives and Endpoints

Secondary objectives	Endpoints
To determine the extent and longevity of 1RG-CART engraftment based on numbers of transduced T-cells in the peripheral blood.	Estimation of 1RG-CART counts in the peripheral blood by flow cytometry at a range of time points (see Section 8.1.1).
To evaluate anti-tumour activity of 1RG-CART therapy in patients with relapsed/refractory neuroblastoma.	Tumour responses according to Response Evaluation Criteria in Solid Tumours (RECIST) Version 1.1 [53], Immune-Related Response Criteria (irRC) [54], and International Neuroblastoma Response Criteria (INRC) [55]. See Appendices 6, 7 and 8. Progression-free survival and overall survival at 2 years (from first 1RG-CART infusion).

3.1.3 Tertiary Objectives and Endpoints

Tertiary objectives*	Endpoints
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

[redacted]

[redacted]

3.2 Design of the Clinical Trial

This is a two centre, Phase I, first-in-human, open label, dose escalation and expansion trial in patients with relapsed or refractory neuroblastoma. The dose escalation phase of the trial will employ cohorts of at least one patient until evidence of 1RG-CART engraftment, 1RG-CART-related toxicity (other than minor non-specific side effects, e.g. Grade 1-2 infusion-related reactions), and/or anti-tumour efficacy is seen.

It is expected that between 15 and 27 patients will be required to complete this trial, the final number depending on the number of dose levels explored. Approximately 9 to 24 patients will be entered into the dose escalation part of the trial. Further patients will be recruited into the dose expansion part of the trial, to give a total of nine patients treated at the expanded dose level.

Each cohort of one or more patients will receive 1RG-CART given as a slow IV injection or infusion (with a maximal infusion rate of 20 mL/m^2/min) on Day 0. See Table 3 in Section 5.2 for a summary of the planned dose escalation scheme.

The first cohort of patients will receive 1x10^7 1RG-CART/m^2 on Day 0 without any preparative lymphodepleting regimen (Dose Level 1). The second cohort of patients will be given 300 mg/m^2/day of cyclophosphamide on each of Days -4 to -1 (total of four doses), followed by 1x10^7 1RG-CART/m^2 on Day 0 (Dose Level 2).

The third cohort of patients will be given 300 mg/m^2/day of cyclophosphamide each day on Days -7 to -4 (total of four doses) and 25 mg/m^2/day of fludarabine each day on Days -8 to -4 (total of five doses), followed by 1x10^7 1RG-CART/m^2 on Day 0 (Dose Level 3).

The fourth cohort of patients will be given the same conditioning regimen as for Dose Level 3, followed by 1x10^8 1RG-CART/m^2 on Day 0 (Dose Level 4).

If 1RG-CART survival is not considered optimal in earlier dose levels and improved efficacy seems likely with a higher 1RG-CART dose, a fifth dose level may be explored. Patients in this cohort will be given the same conditioning regimen as for Dose Levels 3 and 4, followed by 5-10x10^8 1RG-CART/m^2 on Day 0 (Dose Level 5).

If adequate 1RG-CART survival and anti-tumour efficacy is achieved then subsequent dose levels may be omitted and the expansion phase of the trial may commence. Persistence of transduced T-cells (defined as ≥0.02x10^9/L transduced T-cells in peripheral blood) for at least 2 weeks after infusion in the majority of patients in a cohort is considered an indicator of successful engraftment.

In most patients, it is expected that 1RG-CART will only be given once. However, patients may receive a second dose of 1RG-CART as described in Section 5.4.1.

In the expansion phase, additional patients will be recruited to the most favourable cohort (to give a total of nine patients treated at this dose level) to further evaluate safety, 1RG-CART engraftment and survival, and anti-tumour efficacy.

After administration of the 1RG-CART on Day 0, patients will be monitored daily for the first 14 days, then weekly until Day 42. Follow-up visits will be performed at 2, 3, 4, 5, 6, 9, 12 and 24 months from Day 0 and then annually until the end of the trial.

The End of Trial will be declared once all patients have either withdrawn from the trial or the last patient to receive an infusion of 1RG-CART has completed the 24 month follow-up visit (following their last administration of the 1RG-CART). Once the End of Trial is declared, any patients remaining on study will

be consented and re-enrolled to a follow-on protocol in which they will be followed up for at least 15 years after their last dose of 1RG-CART (see Section 12).

3.3 Patient Treatment Group

Patients with relapsed or refractory neuroblastoma and who fulfil the eligibility criteria will be eligible for the trial. It is expected that all patients will be in the paediatric age range, although there is no upper age limit for the trial.

3.4 Definition of Dose Limiting Toxicity

Toxicity will be graded according to the NCI CTCAE Version 4.02 or trial-specific grading scheme for CRS (Appendix 1).

A DLT is defined as any of the following when considered highly probably or probably treatment-related following the first dose of 1RG-CART:

- Uncontrollable pain attributable to 1RG-CART requiring suicide gene activation in the first two weeks after receiving 1RG-CART.
- Progressive central neurological deficit attributable to 1RG-CART.
- Severe immunological activation syndrome requiring ventilatory or prolonged inotropic support.
- Any other reason for activation of the suicide gene within the first two weeks after receiving 1RG-CART.
- Any other fatal event (Grade 5) or life-threatening event (Grade 4) that cannot be managed with conventional supportive measures or which in the opinion of the Trial Steering Committee (TSC) (see Section 3.6) necessitates dose reduction or other modification to trial treatment to avoid a similar hazard in future patients.

Reporting requirements

- All DLTs must be reported as serious adverse events (SAEs) within 24 hours of site staff becoming aware of them (see Section 9). All DLTs will be notified to the TSC by the Sponsor.
- Although not necessarily considered a DLT, a decision to activate the suicide gene by administration of rituximab at any time should be reported as an SAE to the Sponsor. The TSC will also be informed by the Sponsor (see Section 3.6).

Please note that the definition of DLT is not based on NCI CTCAE criteria alone since Grade 3/4 toxicities are anticipated in relation to immune activation and cytokine release but these events are expected to be manageable and reversible.

For patients receiving a second dose, all significant toxicities (including any event which meets the definition of a DLT) will be taken into account for making dose escalation decisions and in determining the recommended Phase II regimen.

If a change is made to the grade or causality of an adverse event during the trial, the event should be reconsidered against the DLT definitions above. Any change which causes an adverse event to be considered a DLT when it was not previously considered a DLT, or vice versa, should be notified to the Sponsor immediately as this may affect dose escalation decisions.

3.5 Criteria for Administration of Rituximab

In the event that unmanageable toxicity attributable to the 1RG-CART is observed, rituximab can be used to eliminate the cells and ameliorate the toxicity. A single dose of 375 mg/m^2 should be administered. This may be repeated if the first dose produces insufficient depletion of 1RG-CART to control toxicity.

It should be noted that rituximab infusions are associated with reactions that are thought to be due to cytokine release. Moreover, rituximab causes B-cell depletion and could therefore exacerbate or prolong the immune suppression caused by fludarabine and cyclophosphamide lymphodepletion. Finally, depletion

of 1RG-CART by rituximab might lead to loss of tumouricidal benefit. The decision to use rituximab should therefore not be made lightly.

The following toxicities are considered reasonable indications for suicide gene activation:

- Pain syndrome not controllable by maximal analgesia (see Appendix 9).
- Severe (Grade 3-4) progressive CNS deficit attributable to 1RG-CART.
- Severe (Grade 3-4) immunological activation syndrome, which cannot be managed with steroids and/or cytokine inhibitors such as tocilizumab or etanercept.
- Progressive pathological clonal T-cell lymphoproliferative disorders.
- Any other severe, unexpected toxicities attributable to 1RG-CART that cannot be managed with conventional measures.

A decision to activate the suicide mechanism by administration of rituximab at any time should be reported as an SAE to the Sponsor. The TSC will also be informed by the Sponsor (see Section 3.6). The Investigator's reasons/justification for rituximab use will also be collected in the electronic case report form (eCRF).

3.6 Trial Steering Committee

A TSC will be convened to review safety and efficacy data from the trial on an ongoing basis, in consultation with the Chief Investigator (CI) and representatives from the Sponsor (including the Medical Advisor). The TSC will provide independent expert advice in the event of any concerns around toxicity and will review safety and efficacy data prior to initiation of a new cohort, in consultation with the CI and Sponsor. The committee will include one member of CRUK's Protocol and Safety Review Board (PSRB) and at least two independent experts with expertise in CAR T-cell therapy and/or other relevant intensive therapies for paediatric patients with advanced malignancy. The PSRB will be informed of the TSC's recommendations.

Amongst other possible scenarios, the TSC will consider late (>2 weeks after administration of 1RG-CART) activation of the suicide mechanism using rituximab and whether this should be considered a DLT or not (see Section 3.4). If it is not considered a DLT, the TSC will make recommendations to modify the protocol or continue unchanged, as appropriate. They will also make recommendations on cohort expansion or dose de-escalation following the occurrence of DLTs of any kind (including DLTs based on activation of the suicide mechanisms using rituximab) (see Section 5.2). Where required, the TSC will make recommendations regarding whether patients should receive a second dose of 1RG-CART and (where applicable) what regimen these patients should receive (see Section 5.4.1). Finally, the TSC will be responsible for recommending early termination of the trial for safety or efficacy reasons.

3.7 Definition of Recommended Phase II Regimen

The recommended Phase II regimen (RP2R) will be based primarily on safety data and on the frequency, persistence and phenotype of 1RG-CART in the peripheral blood. Persistence of transduced T-cells (defined as $\geq 0.02 \times 10^9$/L transduced T-cells in peripheral blood) for at least 2 weeks after infusion in the majority of patients in a cohort will be considered an indicator of successful engraftment. Evidence of anti-tumour efficacy will also be of relevance in defining a Phase II regimen.

3.8 Patient Evaluability

1RG-CART survival

All patients who complete their assigned treatment regimen (first administration of 1RG-CART with/without lymphodepletion*) and provide at least one post-treatment blood sample on or after Day 14 for 1RG-CART analysis, will be evaluable for 1RG-CART survival.

**Patients who do not receive the full target number of 1RG-CART (1×10^7 cells/m^2 at Dose Levels 1-3, 1×10^8 cells/m^2 at Dose Level 4, and $\geq 5 \times 10^8$ cells/m^2 at Dose Level 5 [see Section 5.2 for minimum target number criteria]) and patients who have some or all of their assigned lymphodepleting regimen omitted because of lymphopenia, pancytopenia, infection or similar reason (see Section 5.3.1) will still be evaluable for 1RG-CART survival.*

Feasibility of 1RG-CART administration
All patients entered into the trial (i.e. enrolled for leukapheresis/venepuncture) will be evaluable for feasibility of using 1RG-CART to treat patients with relapsed or refractory neuroblastoma. This includes two main components: the feasibility of T-cell transduction with the MP10413 retrovirus, and the feasibility of treating the patients.

Safety and dose escalation
All patients who are given 1RG-CART or one dose of the lymphodepleting regimen will be evaluable for safety and dose escalation decisions.

Tumour response
All patients who are given one dose of 1RG-CART and who complete a baseline and at least one post-treatment disease assessment will be evaluable for tumour response. Patients who are given one dose of 1RG-CART and experience clear disease progression without a formal post-treatment disease assessment will be considered non-responders.

Overall survival
All patients who receive 1RG-CART will be evaluable for overall survival, regardless of whether they subsequently receive other anti-cancer treatments.

Progression-free survival
All patients who receive 1RG-CART and complete a baseline and at least one post-treatment disease assessment (or experience clear disease progression without a formal post-treatment disease assessment) will be evaluable for progression-free survival, regardless of whether they subsequently receive other anti-cancer treatments.

4 PATIENT SELECTION

4.1 Eligibility Criteria

Patients must fulfil the eligibility criteria listed in Sections 4.1.1 and 4.1.2.

4.1.1 Eligibility Criteria for Leukapheresis/Venepuncture

4.1.1.1 Inclusion Criteria

1. Written informed consent* for leukapheresis/venepuncture and transduction of T-cells.
2. Suitability for leukapheresis/venepuncture defined as:
   - Negative for human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV) 1, HTLV 2, syphilis and hepatitis B
   - Minimum T-lymphocyte count of $0.25x10^9$/L
3. Relapsed or refractory neuroblastoma (the patient must have evidence of active disease even if they do not currently require active treatment).
4. Patients must have at least one lesion that can be evaluated for response by imaging and/or diffuse bone marrow infiltration.
5. Adequate renal function, defined as a glomerular filtration rate (GFR) ≥ 30 mL/min/1.73m^2 (corrected).
6. Karnofsky score ≥60% if ≥16 years old (Appendix 4) or Lansky performance score of ≥60% if <16 years old (Appendix 3).

*Informed consent from the patient's parent or legal guardian is required for all patients under 16 years of age. Patients under 16 years of age may also provide written assent to take part in the trial.

4.1.1.2 Exclusion Criteria

Patients should not meet (or be anticipated to meet) any of the exclusion criteria for the main trial, see Section 4.1.2.2.

4.1.2 Eligibility Criteria for the Main Trial

4.1.2.1 Inclusion Criteria:

1. Histologically proven neuroblastoma, which is relapsed or refractory to conventional treatment.
2. Patients must have at least one lesion that can be evaluated for response by imaging and/or diffuse bone marrow infiltration.
3. Aged ≥12 months at the time written consent is given for the dose escalation phase or aged ≥6 months at the time written consent is given for the dose expansion phase of the trial.
4. Life expectancy of at least two months.
5. Karnofsky score ≥60% if ≥16 years old (Appendix 4) or Lansky performance score of ≥60% if <16 years old (Appendix 3).
6. Adequate renal function, defined as a GFR of ≥30 mL/min/1.73m^2 (corrected).
7. Written (signed and dated) informed consent to the main trial* and be capable of co-operating with treatment and follow-up.

*Informed consent from the patient's parent or legal guardian is required for all patients under 16 years of age. Patients under 16 years of age may also provide written assent to take part in the trial.

Note:

- Evaluation of GD2 expression prior to trial entry is desirable in all patients. Therefore, whenever possible GD2 expression should be checked prior to study entry on a tumour sample taken after relapse/progression. However, as expression of this ganglioside is ubiquitous in neuroblastoma and is not down-regulated in response to therapy [59, 60], re-biopsy is not mandatory for patients with inaccessible tumour sites or in whom GD2 assessment would not be feasible (for example, patients with bone-only disease). GD2 expression will also be assessed in any tumour tissue obtained during the trial (e.g. in patients undergoing bone marrow assessment).
- There are no haematological or biochemical entry requirements for this trial (other than the minimum lymphocyte count required for leukapheresis) since these parameters are frequently deranged in patients with advanced neuroblastoma and would not prevent a patient receiving 1RG-CART. All patients will receive full supportive care, including transfusion of blood products to maintain adequate haematological parameters.

4.1.2.2 Exclusion Criteria:

1. Patients who have received anti-GD2 antibody treatment within the previous 2 weeks (based on the half life of ch14.18 antibody being 1-3 days in children); patients who have received dinutuximab or other anti-GD2-directed antibody may need a longer wash-out period.
2. Patients for whom rituximab is contraindicated due to severe previous hypersensitivity or any other reason.
3. Patients must have recovered from the acute reversible effects of any previous therapy before infusion of the 1RG-CART.
4. Current CNS involvement (including intradural meningeal involvement). Patients who previously had CNS involvement but have been surgically treated and disease free for ≥2 months are eligible.
5. Co-existing chronic progressive neurological disease.
6. Airway compromise by direct tumoural invasion or compression.
7. Patients with active autoimmune disease requiring systemic treatment.
8. Patients who are taking or likely to require high dose systemic corticosteroids or other immunosuppressive therapy (patients on steroid replacement therapy are eligible).
9. Patients at high medical risk because of non-malignant systemic disease including active uncontrolled infection.
10. Major surgery from which the patient has not yet recovered.
11. Female patients who are able to become pregnant (or already pregnant or lactating). However, those patients who have a negative serum or urine pregnancy test before enrolment and agree to use two highly effective forms of contraception (oral; injected or implanted hormonal contraception and condom; have an intra-uterine device and condom; diaphragm with spermicidal gel and condom) effective at the first administration of the lymphodepleting regimen or at administration of the 1RG-CART (whichever comes first), throughout the trial and for six months afterwards are considered eligible. Note that for female patients who receive cyclophosphamide or rituximab, the contraceptive period should be extended to 12 months after cyclophosphamide/rituximab administration.
12. Male patients with partners of child-bearing potential (unless they agree to take measures not to father children by using one form of highly effective contraception [condom plus spermicide] effective at the first administration of the lymphodepleting regimen or at administration of the 1RG-CART [whichever comes first], throughout the trial and for six months afterwards). Men with pregnant or lactating partners must be advised to use barrier method contraception (for example: condom plus spermicidal gel) to prevent exposure to the foetus or neonate.
13. Known to be serologically positive for hepatitis B, hepatitis C or HIV.
14. Any other condition which in the Investigator's opinion would not make the patient a good candidate for the clinical trial.
15. Is a participant in another clinical trial of an investigational medicinal product (CTIMP). Participation in an observational trial or in the follow-up phase of a CTIMP would be acceptable.

4.2 Patient Enrolment

Informed consent from the patient's parent or guardian is required for all patients under 16 years old. Written or verbal assent will be sought from all patients under 16 years old where appropriate. Informed consent is required for all patients aged 16 or older. All patients who provide informed consent or for whom informed consent has been provided and are eligible for leukapheresis/venepuncture must be added to the electronic data capture (EDC) system by the site staff. Screen failure patients who do not go on to be enrolled for leukapheresis/venepuncture should not be added to the EDC system; their details must be added to the screening log.

Before enrolling the patient in the trial, the Investigator or designated representative should determine the eligibility of the patient during the trial screening period. Please ensure that the Sponsor is notified of any eligibility concerns at least four working days before leukapheresis/venepuncture or treatment is planned.

Eligible patients must be enrolled in the EDC system by site staff and then registered by the Sponsor before they undergo leukapheresis/venepuncture and again before they start trial treatment. If the patient is eligible a patient number will be allocated by the EDC system during the initial enrolment process. This patient number will be kept throughout the trial. The Sponsor will send confirmation of the patient registration to the Investigator following enrolment of the patient at each stage. Once the patient's eligibility for the main trial is confirmed, the Sponsor will send confirmation of the patient's assigned dose regimen.

5 TREATMENT

5.1 Dosing Schedule/Treatment Schedule

5.1.1 Leukapheresis/Venepuncture

Patients who meet the eligibility criteria for leukapheresis/venepuncture (see Section 4.1.1) and consent to leukapheresis/venepuncture will have their T-cells collected, transduced and the transduced cells frozen for use in the main trial.

A non-mobilised, steady state, two blood volume apheresis will be performed according to standard local practice to harvest sufficient peripheral blood T-cells for transduction. Alternatively, if feasible, peripheral blood T-cells will be extracted from a sample of whole blood obtained by venepuncture. It is expected that each patient will only undergo a single leukapheresis/venepuncture. However, it is possible a second leukapheresis/venepuncture might need to be conducted in case of poor yield or failure of 1RG-CART manufacture. In addition, a second leukapheresis and manufacture may be performed for patients in Dose Level 1 who had a 'failed' first dose, if they meet the criteria for a second dose but have insufficient stored 1RG-CART to administer the dose required for the currently enrolling dose level.

Those patients who then meet the eligibility criteria for the trial (see Section 4.1.2) and consent to participate in the main trial will be enrolled and treated as described in Sections 5.1.2 and 5.1.3.

5.1.2 Lymphodepletion

Patients treated at Dose Levels 2 to 5 will be given a lymphodepleting regimen prior to administration of the 1RG-CART, as follows:

- Dose Level 2 patients will be given 300 mg/m^2/day of cyclophosphamide on each of Days -4 to -1 (total of four doses), followed by 1RG-CART on Day 0.
- Dose Levels 3 to 5: patients will be given 300 mg/m^2/day of cyclophosphamide on each of Days -7 to -4 (total of four doses) and 25 mg/m^2/day of fludarabine on each of Days -8 to -4 (total of five doses), followed by 1RG-CART on Day 0 (or on Day 0 and Day 1, for patients requiring a split dose).

For patients receiving cyclophosphamide, it will be given by IV infusion with:

- 2 hours pre hydration of glucose 2.5%/sodium chloride 0.45% at 125 mL/m^2/hr with mesna equal to 20% of cyclophosphamide dose
- 6 hours post hydration of glucose 2.5%/sodium chloride 0.45% at 125 mL/m^2/hr with mesna equal to 100% of cyclophosphamide dose.

Fludarabine will be given by IV infusion over 30 minutes in sodium chloride 0.9%. For patients with renal impairment (GFR 30-60 mL/min/1.73m^2 [corrected]), the dose of fludarabine should be reduced according to routine clinical practice (generally by 25%). On days when patients receive both cyclophosphamide and fludarabine, the fludarabine will be given first.

5.1.3 Administration of 1RG-CART

If the 1RG-CART manufacture is successful and sufficient transduced T-cells are obtained, they will be given as a slow IV injection or infusion (with a maximal infusion rate of 20 mL/m^2/min) on Day 0.

If 1RG-CART survival is not considered optimal in Dose Levels 1 to 4 and improved efficacy seems likely with a higher 1RG-CART dose, a fifth dose level may be explored. Patients at Dose Level 5 will receive a higher dose of 1RG-CART (5-10x10^8 1RG-CART/m^2) combined with the same preparative regimen used in Dose Levels 3 and 4. In order to administer higher doses of 1RG-CART, it may be necessary to split the dose over two consecutive days (Day 0 and Day 1) to comply with the daily limits on DMSO administration.

If adequate 1RG-CART survival and anti-tumour activity is achieved at any dose level, then subsequent dose levels may be omitted and the expansion phase of the trial may commence. Persistence of

transduced T-cells (defined as ≥0.02x10^9/L transduced T-cells in peripheral blood) for at least 2 weeks after infusion in the majority of patients in a cohort is considered an indicator of successful engraftment.

In the expansion phase, additional patients will be recruited to the most favourable cohort (to give a total of nine patients treated at this dose level) to further evaluate safety, 1RG-CART engraftment and survival, and anti-tumour efficacy. The most favourable dose level will be determined after a review of all the available data by the TSC, CI and Sponsor.

All patients will receive full supportive care, including pre-medication with antihistamine (doses in accordance with product licence) 30 minutes before T-cell infusion, and IV fluids (125 mL/m^2/hour) from 6 hours prior to the T-cell infusion until 24 hours post infusion. Transfusions of blood products, antibiotics, analgesics, and intensive care (including ventilation, dialysis and other intensive supportive care measures) will also be provided as clinically indicated. Prophylaxis for tumour lysis syndrome is not planned for patients in this trial since tumour lysis syndrome rarely occurs in patients with neuroblastoma. However, urate levels will be monitored and treatment started if indicated.

In most patients, it is expected that 1RG-CART will only be given once. However, patients may receive a second dose of 1RG-CART as described in Section 5.4.1.

5.2 Dose Escalation Scheme

The dose escalation phase of the trial will employ a modified rolling six design [1]. However, cohorts of at least one patient are allowed until there is evidence of 1RG-CART engraftment, 1RG-CART-related toxicity (other than minor non-specific side effects such as Grade 1-2 infusion-related reactions), and/or anti-tumour efficacy is seen. As soon as evidence of 1RG-CART engraftment, 1RG-CART-related toxicity (other than minor non-specific side effects such as Grade 1-2 infusion-related reactions), and/or anti-tumour efficacy is seen, the trial will revert to a minimum cohort size of three patients (expanding to six patients if needed to further evaluate the safety profile, see Section 5.2.2). In case of uncertainty (such as treatment of a single patient with very high or very low disease burden at baseline, or transient low-level 1RG-CART survival and proliferation), one or more additional patients will be enrolled in a cohort. Up to six evaluable patients will be entered at any dose level to determine the safety and feasibility of 1RG-CART therapy before further dose escalation or initiation of the dose expansion phase of the trial.

Although described as ‘dose escalation’, the dose of 1RG-CART will not be escalated in Dose Levels 1 to 3. Instead, it is the conditioning regimen (cyclophosphamide ± fludarabine) which is escalated. At Dose Levels 4 and 5, the conditioning regimen remains the same as for Dose Level 3 whilst the dose of 1RG-CART is escalated.

The dose escalation scheme is summarised in Table 3 (more detailed information on the dosing schedule can be found in Section 5.1). Note that a range is provided for the 1RG-CART cell dose in Dose Level 5. The exact dose of 1RG-CART within this range will be decided based on emerging data from the previous cohorts, and the availability of sufficient cells for each patient. For example, if no engraftment is seen at Dose Levels 1-4, a higher dose of 1RG-CART (10 x10^8 cells/m^2, i.e. 1 x10^9 cells/m^2) might be considered appropriate for patients at Dose Level 5. Conversely, if some engraftment is seen at Dose Level 4 but the extent or duration of engraftment is considered suboptimal, a dose of 5 x10^8/m^2 may be considered sufficient for patients treated at Dose Level 5.

Table 3: Planned Dose Escalations

		Conditioning regimen	
Dose Level	**Dose of 1RG-CART* (Day 0)**	**Cyclophosphamide (300 mg/m^2/day)**	**Fludarabine (25 mg/m^2/day)**
1	1x10^7 cells/m^2	None	None
2	1x10^7 cells/m^2	Day -4 to Day -1 (total of four doses)	None
3	1x10^7 cells/m^2	Day -7 to Day -4 (total of four doses)	Day -8 to Day -4 (total of five doses)

4	$1x10^8$ cells/m^2	Day -7 to Day -4 (total of four doses)	Day -8 to Day -4 (total of five doses)
5	$5\text{-}10x10^8$ cells/m^2	Day -7 to Day -4 (total of four doses)	Day -8 to Day -4 (total of five doses)

*Target cell numbers for transduced cells. Actual number may differ and will be recorded in the electronic case report form (eCRF).

The first patient at any dose level will be observed for toxicity for at least 21 days from Day 0 before a second patient can begin treatment or a decision can be made to dose escalate (or not). If a third patient is to be treated at a dose level, the previous patient must be observed for toxicity for 14 days from Day 0 before they can begin treatment. If further patients are recruited into a cohort, they should not be dosed on the same day and should ideally be dosed at least four days apart. If patients show evidence of engraftment and/or 1RG-CART-related toxicity, a longer period of observation may be necessary before the next patient can begin treatment.

In certain circumstances, dose escalation can be considered after one patient has been followed for at least 21 days after administration of 1RG-CART. However, as dose escalation proceeds and evidence of engraftment is detected, at least three patients will be required at each dose level. The use of single patient cohorts is intended to reduce the number of patients potentially exposed to ineffective regimens. Decisions will be based on the number of DLTs (and any other drug-related AEs) observed at the current dose level, the number of patients enrolled who are at risk of developing a DLT (see Section 3.4), and evidence of 1RG-CART engraftment and activity. When sufficient data are available to assess these, dose escalation may be considered as follows:

- SINGLE-PATIENT COHORTS: If data are only available for one new* patient at a dose level, dose escalation may take place if this patient has received the planned regimen⁺ and been followed for at least 21 days without evidence of 1RG-CART engraftment (1RG-CART <$0.02x10^9$/L at Day 14 with no evidence of increasing peripheral blood CAR T-cell numbers within the first 14 days), 1RG-CART-related toxicity (other than minor non-specific side effects such as Grade 1-2 infusion-related reactions), or anti-tumour efficacy. The patient must also be considered a reasonable candidate for 1RG-CART engraftment (patients with very low disease burden at baseline may not be ideal for assessing engraftment, for example). In case of uncertainty, one or more additional patients must be enrolled. The data from the current cohort must be reviewed by the TSC before the next dose level can open.

- THREE PATIENT COHORTS: If data are available from a minimum of three new* patients treated at a dose level, who have received the planned regimen⁺, and no DLTs have been observed at that dose level, then dose escalation can be considered. Of those three patients, two patients in the cohort must have been treated and followed for at least six weeks and all other patients must have been treated and followed for at least two weeks.

  **Patients undergoing a second infusion of 1RG-CART for any reason cannot be the first patient treated at a new dose level. They will also not count as a 'new' patient for any dose level which requires three patients (they will be considered an 'additional' patient in that cohort).*

  ⁺Patients at Dose Levels 1, 2 and 3 must have received at least $5x10^6$ 1RG-CART/m^2 to be considered to have received the planned regimen. Patients at Dose Level 4 must have received at least $5x10^7$ 1RG-CART/m^2 to be considered to have received the planned regimen. Patients at Dose Level 5 must have received at least $2.5x10^8$ 1RG-CART/m^2 to be considered to have received the planned regimen (or at least $5x10^8$ 1RG-CART/m^2 if the planned regimen is $1x10^9$ 1RG-CART/m^2). At Dose Levels 2 to 5, if a patient does not complete the lymphodepleting regimen due to lymphopenia, pancytopenia, infection or similar reason (see Section 5.3.1), then additional patients will be recruited as necessary to ensure sufficient patients have completed the planned regimen for that cohort before dose escalation can occur. Patients who receive a reduced dose of fludarabine due to renal impairment will be considered to have completed the conditioning regimen as planned. If there is uncertainty about whether an additional patient needs to be recruited the TSC will be consulted.

- If DLT occurs (see Section 3.4), recruitment into that cohort will cease pending discussion by the TSC. If it is agreed that recruitment to the cohort can continue then it will be expanded to include up to six patients. For less severe toxicity or in the case of uncertainty, the cohort may be

expanded up to six patients to better assess the safety profile. Cohort expansion up to six patients may also be appropriate to better assess engraftment and anti-tumour efficacy.

- If it is too soon to take a decision on dose escalation but an eligible patient has been identified who needs to start treatment without further delay, the patient may be enrolled in the current cohort. Up to three additional patients may be enrolled in a cohort (i.e. a total of six patients in a cohort that does not require expansion for toxicity).

Prior to any escalation of the dose level, a dose decision meeting will be held. If possible, the TSC will be present at the same meeting. If this is not possible, the TSC will review the data prior to a dose decision meeting and provide their recommendation. The final decision will be made by the Sponsor and CI in consultation. At the meeting the following data will be reviewed:

- Demographic data.
- Administration data for all IMPs.
- All safety data for patient(s) in the current cohort as described above.
- All available safety data from any patient treated (i.e. data from previous cohorts, and any patients who receive a second dose).
- Any available engraftment and serum cytokine data.

5.2.1 Intra-patient Dose Escalations

Intra-patient dose escalation is not planned in the trial protocol as in general, patients will only receive a single dose of 1RG-CART. However, intra-patient dose escalation may occur in selected patients receiving a second dose of 1RG-CART (see Section 5.4.1).

5.2.2 Expansion of Dose Level(s)

If DLT (as defined in Section 3.4) occurs, recruitment into that cohort will cease pending discussion by the TSC. If it is agreed that recruitment can continue, the cohort will be expanded up to six patients. For less severe toxicity, or in the case of uncertainty, the cohort may be expanded to six patients to better assess the safety profile.

Additional patients will be recruited to the most favourable cohort in the dose expansion phase of the trial, to give a total of nine patients treated at that level.

5.3 Dose Modifications

5.3.1 Dose Reductions

5.3.1.1 1RG-CART

Dose reductions to the 1RG-CART are not allowed, although it is acknowledged that there is a possibility that exact T-cell numbers may fall short of the protocol-described target level. However, if the dose is less than $1x10^6$ 1RG-CART/m^2 administration will not proceed. The dose given to each patient will be documented.

5.3.1.2 Lymphodepleting Regimen

For patients with a low GFR (30-60 mL/min/1.73m^2 [corrected]) the fludarabine dose should be reduced according to routine clinical practice (generally by 25%). Patients who have their fludarabine dose reduced due to low GFR (and have no other dose reductions) will be considered to have completed the planned regimen and will not be replaced (see Section 5.4.2).

Other dose reductions to the conditioning regimen are not planned but may be clinically indicated, for example in the context of lymphopenia, pancytopenia or infection. In this situation, some or all of the lymphodepleting regimen may be omitted as clinically indicated. Any treatment-related toxicities will be

managed with conventional supportive measures. If a patient does not complete the lymphodepleting regimen due to lymphopenia, pancytopenia, infection or similar reason, then they will be replaced (see Sections 5.2 and 5.4.2).

5.3.2 Dose Delays

In the event that the lymphodepleting regimen is interrupted for intercurrent illness or other reason, the patient may complete or recommence the preparative regimen after recovery, according to the Chief Investigator's judgment after consultation with the Sponsor.

If the patient has completed lymphodepleting therapy but is unable to receive the 1RG-CART on Day 0 for any reason, clinical judgement will be used as to whether it is appropriate to delay the administration of 1RG-CART, or whether to wait for the patient's blood counts to recover sufficiently before repeating the lymphodepleting regimen and administering the 1RG-CART. If the patient is deemed unsuitable to receive the 1RG-CART, they will be withdrawn from the trial (see Section 11) and replaced. Similarly, clinical judgment will be used to decide on the best course of action (to omit or administer the dose late) for a patient scheduled to receive a split dose, who is unable to receive the planned dose on Day 1.

In case of uncertainty, individual cases should be discussed with the Sponsor.

5.3.3 Dose Interruptions

In the event of severe infusion reactions the cyclophosphamide/fludarabine/1RG-CART infusion should be stopped and the patient treated as clinically indicated. When the patient has recovered the infusion may be restarted. For severe life-threatening toxicity, as determined by the clinical judgement of the Investigator, the infusion will be permanently discontinued and there will be no rechallenge. For less severe toxicity, the infusion will be restarted on recovery and given at a reduced rate following administration of hydrocortisone and chlorpheniramine, in accordance with local practice.

If an infusion is interrupted for mechanical, technical or any other reason, then this should be dealt with according to local practice and the infusion restarted as soon as possible.

5.4 Duration of Treatment

In most patients, it is expected that 1RG-CART will only be given once. However, patients may receive a second dose of 1RG-CART as described in Section 5.4.1. The conditioning regimen and/or 1RG-CART dose for these patients may differ from the first dose depending on the patients' circumstances (see Section 5.4.1 for details).

Patients at Dose Level 2 will receive cyclophosphamide once daily for four days prior to administration of the 1RG-CART.

Patients at Dose Levels 3 to 5 will receive cyclophosphamide once daily for four days and fludarabine once daily for five days prior to administration of the 1RG-CART.

In the event that the patient is lymphopenic or pancytopenic at trial entry or becomes excessively lymphodepleted before completing their assigned lymphodepletion regimen, the Investigator may omit some or all of the lymphodepleting regimen, as clinically indicated (see Section 5.3.1.2).

After administration of the 1RG-CART on Day 0, patients will be monitored daily for the first 14 days, then weekly until Day 42. Follow-up visits will then be performed at 2, 3, 4, 5, 6, 9, 12 and 24 months from Day 0. Patients will then be assessed annually until the End of Trial is declared, at which time any patients still on study will be consented and re-enrolled to a follow-on protocol (see Section 12).

5.4.1 Retreatment of Patients

It is expected that most patients will receive a single infusion of 1RG-CART. However, patients may receive a second dose as follows:

- **After a 'failed' first dose**; i.e. patients who show evidence of minimal 1RG-CART survival and function (manifest as transient or no 1RG-CART detectable in the peripheral blood with no evidence of anti-tumour efficacy and minimal toxicity). These patients are likely to have received ineffective therapy and if they meet the criteria in Section 5.4.1.1, they may receive a second dose of 1RG-CART with a different conditioning regimen and/or a higher dose of 1RG-CART (as for the currently enrolling cohort).
- **After a 'successful' first dose**; i.e. patients who tolerated the first dose of 1RG-CART and showed evidence of 1RG-CART survival with evidence of anti-tumour activity (for at least 8 weeks) but who subsequently show progressive disease in association with declining 1RG-CART levels. These patients may receive a second dose if they meet the criteria in Section 5.4.1.2. They will generally receive the same 1RG-CART dose and conditioning regimen as originally given, unless there is clear evidence from other completed cohorts that superior results are expected with a different dose/regimen (which could be a lower or higher cohort than the patient originally entered).
- Patients who do not fall into either of the first two categories but meet the criteria in Section 5.4.1.3 and whom, in the opinion of the Investigator, may benefit from another dose will be reviewed by the TSC, CI, PI (where applicable) and the Sponsor. These patients will only receive a second dose if it is agreed by the TSC, CI, PI (where applicable) and the Sponsor that it is in the patient's best interest. The conditioning regimen and dose of 1RG-CART the patient will receive will be agreed by the TSC, CI, PI (where applicable) and Sponsor.

5.4.1.1 Patients who have received ineffective therapy (a 'failed' first dose)

It is anticipated that survival and function of 1RG-CART may be inadequate in some patients (a 'failed' first dose) and that in these patients it may be appropriate to administer a second dose of 1RG-CART with a different conditioning regimen and/or higher dose of cells. Accordingly, a second dose of 1RG-CART may be given if the patient meets the following criteria:

- The patient has adequate cryopreserved 1RG-CART available for a second dose of 1RG-CART. Except for patients enrolled in Dose Level 1, for these patients a second manufacture may be performed if they have insufficient cryopreserved 1RG-CART to be treated in the currently enrolling dose level.
- The patient still fulfils the trial entry criteria for Lansky/Karnofsky performance status and renal function required to tolerate another infusion.
- There are no alternative therapies available that are considered more likely to benefit the patient.
- The patient tolerated the first infusion with minimal toxicity during a follow-up period of six weeks.
- The patient has low levels ($<0.02 \times 10^9$/L) or undetectable circulating 1RG-CART at or after Day 14 and for at least 3 weeks prior to the second infusion.
- There was no clinical evidence of anti-tumour activity following the first 1RG-CART infusion.

For patients in Dose Level 1 requiring a second leukapheresis and manufacture to receive a second dose in the currently enrolling dose level, the patient must also meet the following eligibility criteria for leukapheresis/venepuncture:

- The patient is negative for HIV, HTLV1, HTLV2, syphilis and hepatitis B.
- The patient has a minimum T-lymphocyte count of 0.25×10^9/L.
- The patient has no detectable circulating 1RG-CART prior to leukapheresis/venepuncture.

Patients fulfilling these criteria at the Day 42 ('End of treatment') assessment may receive a second dose of 1RG-CART according to the currently enrolling cohort/dose level. They will be considered "additional" patients in that dose level and cannot be the first patient treated at that dose level. The second dose should be given at least 6 weeks after the first dose (i.e. on or after Day 42). If there is uncertainty about whether a patient is eligible for a second dose, the TSC will be consulted. Patients may receive palliative anti-cancer therapy at the investigator's discretion, while awaiting a decision or timeslot for administration of a second dose.

5.4.1.2 Patients who benefited from the first dose (a 'successful' first dose)

Patients who tolerated the first dose of 1RG-CART and showed evidence of 1RG-CART survival with evidence of anti-tumour activity for at least 8 weeks may receive a second dose if they fulfil the following requirements:

- The patient has adequate cryopreserved 1RG-CART available for a second dose of 1RG-CART.
- The patient tolerated the first infusion without dose-limiting or other severe or unmanageable toxicity for a follow-up period of at least six weeks.
- The patient still fulfils the trial entry criteria for Lansky/Karnofsky performance status and renal function required to tolerate another infusion.
- There are no alternative therapies available that are considered more likely to benefit the patient.
- There was objective clinical evidence of anti-tumour activity following the previous 1RG-CART infusion, i.e. a CR, PR, tumour shrinkage, or stable disease for at least 8 weeks.
- The patient now has evidence of progressive disease in the context of declining 1RG-CART. Levels of circulating 1RG-CART must be low ($<0.02x10^9$/L) or undetectable for at least 3 weeks prior to the second infusion.
- The patient has not received any alternative systemic anti-cancer therapy since the first 1RG-CART infusion (palliative radiotherapy and/or corticosteroids for symptom control are allowed).

Patients undergoing a second 1RG-CART infusion should receive the same conditioning regimen (or lack of) and dose of 1RG-CART as in their first treatment cycle, unless data from subsequent completed dose levels clearly indicates that improved outcomes are likely with a different regimen. If they are treated with any regimen other than their original regimen, they will be considered "additional" patients in that dose level. The dose level they enter for their second dose should be complete (i.e. the required number of patients must have completed the required observation period following treatment and the data from the cohort must have been reviewed by the TSC).

The decision to re-treat a patient, and whether the patient should receive a different lymphodepletion regimen and/or dose of 1RG-CART or not, will be made by the CI and Sponsor in consultation with the TSC.

5.4.1.3 Patients not having a 'Failed' or 'Successful' First Dose

Some patients may not meet the criteria for a 'failed' (Section 5.4.1.1) or 'successful' (Section 5.4.1.2) first dose if there are manageable signs of toxicity suggestive of engraftment, some low level engraftment ($<0.02x10^9$ 1RG-CART/L) or a short lived clinical response (<8 weeks in duration). The PI/CI may, however, consider it to be in the patient's best interest for a second dose to be given.

In these circumstances, if the patient has sufficient transduced T-cells leftover from their first manufacture, the PI/CI may request the patient receive a second dose. Such patients may receive a second infusion of their own stored 1RG-CART if the TSC, CI, PI (where applicable) and Sponsor agree it is in the patient's best interest and they fulfil the following requirements:

- The patient has adequate cryopreserved 1RG-CART available for a second dose of 1RG-CART.
- The patient tolerated the first infusion without dose-limiting or other severe or unmanageable toxicity for a follow-up period of at least six weeks.
- The patient still fulfils the trial entry criteria for Lansky/Karnofsky performance status and renal function required to tolerate another infusion.
- There are no alternative therapies available that are considered more likely to benefit the patient.
- The patient has low levels ($<0.02x10^9$/L) or undetectable circulating 1RG-CART prior to the second infusion.

The following data will be reviewed: medical history, diagnosis, demographic data, adverse events (including serious adverse events), IMP administration, cytokine and engraftment data, and results of any

disease assessments (bone marrow, scan results). Other data may also be reviewed as required on a case by case basis.

The conditioning regimen and dose of 1RG-CART for these patients will be agreed by the TSC, CI, PI (where applicable) and Sponsor on a case by case basis. If they are treated with any regimen other than their original regimen, they will be considered "additional" patients in that dose level and cannot be the first patient treated at that dose level.

5.4.2 Replacement of Patients

Any patient meeting the following criteria will be replaced by another patient treated at the same dose level:

- Withdrawal from the trial before two weeks from first administration of 1RG-CART for reasons other than treatment-related toxicity.

- Failure to complete the assigned treatment regimen (first administration of 1RG-CART with/without lymphodepletion). Patients who receive a reduced dose of fludarabine due to low GFR (and have no other dose reductions) are considered to have completed the assigned regimen and do not need to be replaced. Patients who have a dose reduction due to lymphopenia, pancytopenia, infection or similar reason will be replaced to ensure that sufficient patients in the cohort have completed the assigned lymphodepleting regimen prior to dose escalation (see Section 5.2).

- Treatment with significantly fewer 1RG-CART than the target cell number for that cohort (patients at Dose Levels 1, 2 and 3 must have received at least $5x10^6$ 1RG-CART/m^2 to be considered to have received the planned regimen; patients at Dose Level 4 must have received at least $5x10^7$ 1RG-CART/m^2 to be considered to have received the planned regimen; and patients at Dose Level 5 must have received at least $2.5x10^8$ 1RG-CART/m^2 [or at least $5x10^8$ 1RG-CART/m^2 if the planned regimen is at least $1x10^9$ 1RG-CART/m^2] to be considered to have completed the planned regimen).

5.5 Concomitant Medication and Treatment

Concomitant medication may be given as medically indicated. Details (including start and stop dates) of the concomitant medication/treatment given must be recorded in the patient's medical records and details entered into the electronic case report form (eCRF).

Standard drugs required by the patient may be administered alongside the trial protocol.

No herbal, homeopathic agents or food supplements will be allowed between Day -10 and Day 20 following T-cell infusion, unless recommended by the Principal Investigator (PI).

All patients will receive full supportive care, including pre-medication with antihistamine (dose in accordance with product licence) 30 minutes before T-cell infusion, and IV fluids (125 mL/m^2/hour) from 6 hours prior to the T-cell infusion until 24 hours post infusion. Transfusions of blood products, antibiotics, analgesics, antiepileptics and intensive care (including ventilation, dialysis and other intensive supportive care measures) will also be provided as clinically indicated. Details of these should be recorded in the eCRF. CRS will be managed according to local policy. A scheme for managing severe cytokine release syndrome is provided in Appendix 2. This should be considered only as a guide and deviations from this scheme are allowed according to the Investigator's judgement.

The use of immunosupressants, such as high dose corticosteroids, should be avoided where possible as these are likely to influence the efficacy and possibly safety of 1RG-CART. Patients on low dose corticosteroids, for example for asthma, should ideally be weaned off them before entering the trial (corticosteroids for adrenal replacement therapy are allowed and should be continued).

Radiotherapy may be given concomitantly for the palliative control of pain. Irradiated lesions will not be evaluable for response.

In general, patients should not receive other anti-cancer therapy (including radiotherapy except as above) or investigational drugs for the first six weeks after administration of the 1RG-CART. However, patients with clear failure of engraftment may receive palliative anti-cancer therapy at the Investigator's discretion, while awaiting a decision or timeslot for administration of a second dose of 1RG-CART. Administration of other systemic anti-cancer therapy at any time will be considered an indicator of treatment failure (progressive disease). However, palliative radiotherapy will be allowed for symptom control without necessarily indicating progressive disease.

Patients who have been administered 1RG-CART and subsequently require alternative anti-cancer therapy of any kind should remain on trial (it is not a reason for withdrawal – see Section 11) and continue to be monitored as described in Section 7. Details of the anti-cancer therapy must be recorded in the patient's medical records and details entered into the eCRF.

6 PHARMACEUTICAL INFORMATION

1RG-CART is an autologous genetically modified cell therapy. T-cells to be used in the manufacture of 1RG-CART will be sourced from each patient at GOSH as part of the trial procedures.

GOSH shall establish and maintain a system for patient and product traceability. All patient identifiers need to match those issued by CRUK following patient registration. Upon receipt of the bag containing whole blood or leukapheresate, a unique batch number will be assigned which will link the derived cell product with the Batch Manufacturing Record and to the initial patient donation. This enables tracking of the patient and 1RG-CART as required under the ATMP regulations.

6.1 1RG-CART

6.1.1 Supply of 1RG-CART

A complete certificate of analysis and a Qualified Person (QP) certification must be provided to CRUK with each batch of the advanced therapy investigational medicinal product (ATIMP) 1RG-CART and copies be retained in the

1RG-CART will be manufactured to EU Good Manufacturing Practice and supplied by:

Cellular Therapies

Great Ormond Street Hospital for Children NHS Foundation Trust

must send a copy of the IMP Despatch Request Form to the CSM/CRA on despatch of the 1RG-CART.

The primary and, where applicable, the secondary packaging for the 1RG-CART will be labelled according to Eudralex Volume 4: Annex 13 ‘Investigational Medicinal Products’ of the European Union Guide to Good Manufacturing Practice.

An example of the label(s) can be found in the Trial Master File (TMF).

6.1.2 Pharmaceutical Data

6.1.2.1 Formulation of 1RG-CART

1RG-CART are autologous, patient-derived MP10413 retrovirus transduced T-cells cryopreserved in one or several aliquots

[redacted]

6.1.2.2 Storage Conditions

All supplies must be stored in a secure, limited access storage area.

1RG-CART will be stored in the vapour phase of liquid nitrogen until required for use whereupon they will be thawed, brought to a temperature of 37°C, and administered within 30 minutes of thawing to the patient.

6.1.2.3 Method of Thawing

Frozen 1RG-CART will be thawed at the patients' bedside in accordance with local procedures for infusion of donor lymphocytes.

6.1.2.4 1RG-CART Administration

Good aseptic practice must be employed when preparing 1RG-CART for IV injection/infusion.

Once the patient has been prepared for 1RG-CART administration, the 1RG-CART will be thawed and brought to 37ºC. They must be administered within 30 minutes of thawing as a slow IV injection or infusion (with a maximal infusion rate of 20 mL/m^2/min) through a central venous line. If needed and to avoid exceeding the maximum daily volume that can be administered, the dose may be split and given over two consecutive days.

6.1.2.5 Accidental Spillages

1RG-CART should be treated as a blood product and any spillages should be cleaned up as per local hospital policy.

6.1.3 1RG-CART Accountability

Accurate records of shipment, dispensing and returns for the ATIMP 1RG-CART for intravenous administration must be maintained by [redacted]. This inventory record must be available for inspection at any time by CRA or CSM of the Sponsor. ATIMP supplies are to be used only in accordance with this protocol and under the supervision of the Investigator.

The Investigator undertakes not to destroy any unused or returned ATIMP unless authorised to do so by the Sponsor. Any dispensed ATIMP which is unused must be destroyed according to hospital procedures and properly accounted for using the ATIMP Destruction Form and also on the ATIMP Accountability Record. During the course of the trial the CRA will check the numbers of vials/bags of 1RG-CART dispensed, the number used and the number destroyed or returned. GOSH Cellular Therapies will give an account of any discrepancy.

6.2 Cyclophosphamide and Fludarabine

6.2.1 Supply of Cyclophosphamide and Fludarabine

Cyclophosphamide and fludarabine will be used outside their licensed indications in this trial and are therefore considered IMPs. Both drugs are available commercially. The Investigators will be responsible for

their own supply of cyclophosphamide and fludarabine. Dispensed cyclophosphamide and fludarabine will be labelled by the Pharmacy according to Eudralex Volume 4: Annex 13 'Investigational Medicinal Products' of the EU Guide to Good Manufacturing Practice. The dispensed label applied by Pharmacy will be approved by CRUK and an example of the labels will be filed in the Pharmacy Folder. Sufficient quantities of either cyclophosphamide, fludarabine or both cyclophosphamide and fludarabine will be dispensed to cover the prescribed dose (as per Section 5.1).

Cyclophosphamide and fludarabine are cytotoxics and must be handled with care in accordance with local policy.

Good aseptic practice must be employed when preparing cyclophosphamide and fludarabine solutions for infusion.

6.2.2 Cyclophosphamide

6.2.2.1 Formulation of Cyclophosphamide

Each vial of cyclophosphamide powder for solution for injection contains cyclophosphamide monohydrate as a white crystalline powder equivalent to either 500 mg, 1000 mg or 2000 mg anhydrous cyclophosphamide in type I or III clear glass injection vials with butyl rubber closures and aluminium caps.

Please also refer to the latest Summary of Product Characteristics (SmPC) for the current approved pharmaceutical information.

6.2.2.2 Cyclophosphamide Storage Conditions and Stability

Unopened vials of cyclophosphamide are stable for 36 months from the date of manufacture (refer to expiry date stated on the vial label) when stored at or below 25°C.

6.2.2.3 Cyclophosphamide Reconstitution, Stability and Administration

Cyclophosphamide will only be given intravenously (IV) in this trial; oral administration is not allowed.

Each vial of cyclophosphamide should be dissolved with either water for injection or 0.9% (w/v) aqueous sodium chloride to a concentration of 20 mg/mL. Please refer to CRUK supplied approved Pharmacy Guidelines.

After reconstitution for intravenous (IV) administration, store at 2 to 8°C in accordance with local practice (but for no more than 7 days) and protect from light.

Cyclophosphamide solution for IV administration will be prepared as per CRUK supplied approved Pharmacy Guidelines.

Patients at Dose Level 2 will receive 300 mg/m^2/day of cyclophosphamide by IV infusion on Days -4 to -1 prior to receiving 1RG-CART on Day 0 (see Section 5.1). Patients at Dose Levels 3 to 5 will receive 300 mg/m^2/day of cyclophosphamide by IV infusion on Days -7 to -4 prior to receiving 1RG-CART on Day 0 (see Section 5.1).

Each vial of cyclophosphamide is for single use only. Any unused contents and/or vials dispensed for patient use must be destroyed as per local policies and accounted for as described in Section 6.2.4.

6.2.3 Fludarabine

6.2.3.1 Formulation of Fludarabine

Fludarabine will only be given intravenously (IV) in this trial; oral administration is not allowed.

Fludarabine is available in two formulations for IV use: 25 mg/mL concentrate for solution for injection or infusion and 50 mg powder for solution for injection or infusion; both product formulations contain 50 mg fludarabine phosphate per vial. For example, the solution (25 mg/mL) for injection is presented as a sterile 2 mL fill in a 5 mL type I glass vial with bromobutylic rubber stopper and metallic cap (aluminium) with a polypropylene disk. For a list of excipients present in the solution formulation please refer to the applicable Summary of Product Characteristics (SmPC).

Please also refer to the latest SmPC for the current approved pharmaceutical information for the relevant fludarabine formulation.

6.2.3.2 Fludarabine Storage Conditions and Stability

Unopened vials of fludarabine stored at 2 to 8°C are stable for 2 years. Please refer to the vial label for the expiry date of the product.

6.2.3.3 Fludarabine Dilution, Stability and Administration

Please also refer to the latest SmPC for the current approved pharmaceutical information.

Fludarabine solution for administration will be prepared as per CRUK supplied approved Pharmacy Guidelines.

Fludarabine diluted in 0.9% (w/v) saline is stable for up to 28 days. However, from a microbiological point of view it should be used within 24 hours of being prepared when stored at 2 to 8°C.

Patients at Dose Levels 3 to 5 will receive 25 mg/m^2/day of fludarabine by IV infusion over 30 minutes on Days -8 to -4 prior to receiving 1RG-CART on Day 0 (see Section 5.1).

Each vial of fludarabine is for single use only. Any unused contents and/or vials dispensed for patient use must be destroyed as per local policies and accounted for as described below.

6.2.4 Cyclophosphamide and Fludarabine Accountability

Pharmacy records should be kept of the cyclophosphamide and fludarabine dispensed to trial patients, the expiry date and batch number used should be recorded as well as vials dispensed and returned (if applicable). These records must be available for inspection by CRAs or CSMs of the Sponsor at any time.

6.3 Rituximab (MabThera®)

6.3.1 Supply of Rituximab (MabThera®)

Rituximab (MabThera®) will be used a rescue medication (when necessary) but will be used outside of its licensed indication; it is considered a NIMP in the trial. Rituximab (MabThera®) is available commercially and the Investigators will be responsible for their own supply of rituximab (MabThera®). Dispensed rituximab (MabThera®) will be labelled according to local practice.

6.3.2 Formulation of Rituximab (MabThera®)

Rituximab (MabThera®) concentrate solution for infusion (100 mg/vial or 500 mg/vial) is presented in either a 10 mL or 50 mL type I glass vial with butyl rubber stopper and metallic cap. Excipients present in the formulation are sodium citrate, Polysorbate 80, sodium chloride, sodium hydroxide, hydrochloric acid, water for injection.

6.3.3 Rituximab (MabThera®) Storage Conditions and Stability

Please refer to the vial label for the expiry date of the product.

Unopened vials of rituximab (MabThera®) stored at 2 to 8°C protected from light are chemically stable for 30 months from date of manufacture.

6.3.4 Rituximab (MabThera®) Dilution, Stability and Administration

Good aseptic practice must be used when preparing rituximab (MabThera®) concentrate solution for infusion.

Please also refer to the latest SmPC for the current approved pharmaceutical information.

Rituximab (MabThera®) solution for administration will be prepared as per local procedures.

The prepared infusion solution of rituximab (MabThera®) is physically and chemically stable for 24 hours at 2 to 8°C and subsequently 12 hours at room temperature.

From a microbiological point of view, the prepared infusion solution should be used immediately. If not used immediately, in use storage times and conditions prior to use are the responsibility of the user and would normally not be longer than 24 hours at 2°C to 8°C, unless dilution has taken place in controlled and validated aseptic conditions.

If required, patients will receive a single dose of 375 mg/m^2 of rituximab (MabThera®) administered by slow infusion according to guidance in the SmPC for rituximab (MabThera®) and CRUK supplied approved Pharmacy Guidelines. At the Investigator's discretion, this dose may be repeated if there is insufficient depletion of 1RG-CART and the patient's symptoms fail to resolve or improve sufficiently.

Each vial of rituximab (MabThera®) is for single use only. Any unused contents and/or vials dispensed for patient use must be destroyed as per local policies and accounted for as described below.

6.3.5 Rituximab (MabThera®) Accountability

Pharmacy records should be kept of the rituximab (MabThera®) dispensed to trial patients, the expiry date and batch number used should be recorded as well as vials dispensed and returned (if applicable). These records must be available for inspection by CRAs or CSMs of the Sponsor at any time.

7 INVESTIGATIONS SCHEDULE

In cases where a patient has investigations at a different hospital, for example weekly blood samples, scans and other investigations as appropriate, then it is the Investigator's responsibility to ensure he/she receives and reviews the reported results. These results must be available for source data verification. Laboratory reference ranges, including effective dates, and evidence of laboratory accreditation must be obtained from all laboratories used. For scan results, the original images and reports must be available for comparison to any scan performed at the investigator site and be in a format that is suitable for comparison. For all other investigations, apart from the results, any supporting data must also be made available for source data verification or review.

The Investigator or delegate must inform the Sponsor of any changes to their laboratory normal ranges or to any laboratory accreditation and provide any new documentation.

Blood volumes

European Medicines Agency recommendations for trial related blood loss in paediatric populations are that no more than 3% of total blood volume should be taken during a four week period and not more than 1% of total blood volume at a single time-point. With a total blood volume estimated to be 80 to 90 mL per kilogram (kg) body weight, this equates to 2.4 mL to 2.7 mL blood per kg body weight during a four week period, or 0.8 mL to 0.9 mL blood per kg at any one time. Table 4 shows the total volume of blood by patient weight and the corresponding 1% and 3% blood volumes.

These recommended volumes are likely to be exceeded in this trial in all but the heaviest patients. The relatively high blood volumes required for investigations in this trial are because of the possible toxicities associated with this novel trial treatment, which may be severe, and the need to monitor all patients closely for at least two weeks for evidence of immune activation, cytokine release, and resulting organ dysfunction. Where possible smaller volumes of blood will be taken and all patients will be monitored for anaemia and actively treated as needed.

A number of different samples are taken in this study. The following routine samples are taken to monitor patient safety:

	Volume collected per sample in patients:	
Sample type	**≤ 12 years old**	**13 years and older**
Haematology	0.5 mL	4.0 mL
Biochemistry	1.0 mL	5.0 mL
Coagulation profile	1.4 mL	4.5 mL
CRP / lactate dehydrogenase (LDH)*	1.0 mL	5.0 mL

*Additional volume per sample at time points where no biochemistry sample is taken, otherwise these will be assessed using the sample collected for biochemistry.

The following samples are taken for research purposes (volumes not affected by age):

- [redacted]
- Sample for 1RG-CART quantification (5.0 mL per sample)
- [redacted]
- Cytokine samples (2.0 mL per sample) – research specific safety samples

Total study-related blood loss for this study for a child **≤12 years old** is estimated as follows:

Time period	Volume of blood for safety assessments	Volume of blood for research assessments	Total volume of blood for time period
Day -1 to Day 27*	35.4 mL	99.0 mL	134.4 mL
Day 28, Day 35, Day 42 and 2 month follow-up*	10.2 mL	27.0 mL	37.2 mL
Monthly follow-up (at 3, 4, 5, 6, and 9 months)*	1.5 mL per visit	0.0 to 7.0 mL per visit	1.5 to 8.5 mL per visit
Long term follow-up (at 12 and 24 months)	1.5 mL per visit	5.0 mL per visit	6.5 mL per visit
Long term follow-up (annual visits)	Not applicable	5.0 mL	5.0 mL

*Approximately 4 week period

Over a four week period, the largest total volume collected for a child up to 12 years old will be 134.4 mL. This would be 3% of a total blood volume of 4675 mL, which at 85 mL per kg would be expected for a 55 kg child. 106.5 mL of this 134.4 mL will be collected during the first two weeks of the trial (Day -1 to Day 14) when the patient is hospitalised and being intensely monitored. These volumes do not include blood loss from venepuncture or leukapheresis to obtain T-cells for 1RG-CART production.

Total study-related blood loss for this study for a child **13 years or older** is estimated as follows:

Time period	Volume of blood for safety assessments	Volume of blood for research assessments	Total volume of blood for time period
Day -1 to Day 27*	186.0 mL	99.0 mL	285.0 mL
Day 28, Day 35, Day 42 and 2 month follow-up*	49.5 mL	27.0 mL	76.5 mL
Monthly follow-up (at 3, 4, 5, 6, and 9 months)*	9.0 mL per visit	0.0 to 7.0 mL per visit	9.0 to 16.0 mL per visit
Long term follow-up (at 12 and 24 months)	9.0 mL per visit	5.0 mL per visit	14.0 mL per visit
Long term follow-up (annual visits)	Not applicable	5.0 mL	5.0 mL

*Approximately 4 week period

Over a four week period, the largest total volume collected for a child of 13 years or older will be 285.0 mL. This would be 3% of a total blood volume of 4675 mL, which at 85 mL per kg would be expected for >80 kg child. 242.5 mL of this 285.0 mL will be collected during the first two weeks of the trial (Day -1 to Day 14) when the patient is hospitalised and being intensely monitored. These volumes do not include blood loss from venepuncture or leukapheresis to obtain T-cells for 1RG-CART production. All patients will have frequent blood counts and top-up transfusions as needed (see Section 5.5).

Table 4: Total Blood Volume, 1% and 3% of Total Blood Volume by Weight Assuming 85 mL per kg Total Blood Volume

Weight (kg)	Total Blood Volume (mL)	1% Blood Volume (mL)*	3% Blood Volume (mL)*
5	425	4	12
10	850	8	24
15	1275	12	36
20	1700	17	51
25	2125	21	63
30	2550	25	75
35	2975	29	87
40	3400	34	102
45	3825	38	114
50	4250	42	126
55	4675	46	138
60	5100	51	153
65	5525	55	165
70	5950	59	177
75	6375	63	186
80	6800	68	204

* all blood volumes have been rounded down

Time-points for the pharmacodynamic assays have been aligned in order to minimise invasiveness and reduce the volume of dead space blood that is removed from the patient. Where possible, blood for haematology and biochemistry analysis will be taken at the same times as other samples for the same reasons. Investigators must discuss with the Sponsor any concerns regarding the volume of study-related blood loss for a particular patient.

7.1 Pre-treatment Evaluations

Details of all evaluations/investigations for enrolled patients, including relevant dates, required by the protocol must be recorded in the medical records so that the eCRF can be checked against the source data.

Please also refer to the tabulated Schedule of Assessments in Section 7.9.1.

7.1.1 Obtaining Written Informed Consent

Patients of 16 years of age or older must provide written informed consent for the trial. Parents or guardians of patients under 16 years of age must provide written informed consent. Written or verbal assent will be sought from patients under 16 years of age where appropriate. Information sheets and consent / assent forms will be provided as follows:

- Patients 16 years of age or older (information sheet and consent form)
- Parents/legal guardians of patients under 16 years of age (information sheet and consent form)
- Patients below 16 years of age (information sheet and assent form):

- Patients 13-15 years of age
- Patients 8-12 years of age
- Patients <8 years of age

Written informed consent / assent must be obtained from the patient and / or the patient's parent or guardian before any protocol-specific procedures are carried out. Patients who move from one age bracket to another during the course of the trial will be re-consented / re-assented using the appropriate information sheet and consent/assent form in accordance with local practice.

Consent to evaluate GD2 expression in tumour samples collected prior to study entry (using biopsies taken at the time of disease relapse/progression) will be obtained where possible. Consent for leukapheresis and T-cell transduction will be obtained within 28 days before leukapheresis. The main trial consent will be obtained before any screening investigations for the main trial are carried out. If there are more than 28 days between main trial informed consent and the start of dosing (with whichever IMP [1RG-CART / fludarabine / cyclophosphamide] is given first), then the Investigator should consider whether repeat consent should be obtained from the patient and/or patient's parent or guardian. The patient / patient's parents or guardian must be given adequate time to think about their commitment to the trial.

Only the PI and those Sub-Investigator(s) delegated responsibility by the PI, and who have signed the Delegation Log, are permitted to obtain informed consent from patients or the patient's parent/guardian and sign the consent form. All signatures must be obtained before any medical intervention required by the protocol (ICH GCP 4.8.8 and 8.3.1.2). The date of the signatures of both the patient and/or, patient's parent/guardian, and the PI/Sub-Investigator should be the same.

The PI or the Sub-Investigator must inform the patient and/or their parent/guardian about the background to, and present knowledge of the normal management of their disease and 1RG-CART, and must also ensure that the patient is aware of the following points:

- That the use of 1RG-CART is new and that the exact degree of activity is at present unknown, but that treating him/her will contribute to further knowledge.
- The expected toxicity of 1RG-CART and the possibility of experiencing side-effects.
- The known toxicity of fludarabine and / or cyclophosphamide (where applicable) and the possibility of experiencing side-effects.
- That long-term follow-up is required because it is an ATIMP and this may require enrolment in a subsequent follow-up trial.
- If appropriate, the potential dangers of becoming pregnant (or the patient's partner becoming pregnant) and that he/she has been given information about appropriate medically approved contraception (refer to Section 9.7).
- That he/she may refuse treatment either before or at any time during the trial and that refusal to participate will involve no penalty or loss of benefits to which they are otherwise entitled.
- Who to contact for answers to pertinent questions about the research and their rights, and also who to contact in the event of a research-related injury.

A copy of the informed consent documents (ICD) must be given to the patient and/or their parent/guardian to keep and the original ICD, must be filed in the Investigator Trial File (ITF) (unless otherwise agreed that the original document will be filed in the medical records and copies kept in the ITF) and a copy placed in the patient's medical records.

7.1.2 Evaluations at Baseline/Pre-Trial

7.1.2.1 Within 14 days Prior to Leukapheresis/Venepuncture

The patient should consent to leukapheresis/venepuncture within 28 days before leukapheresis. Their eligibility (as defined in Section 4.1.1) should be confirmed before having their T-cells collected and transduced. The following must therefore be performed/obtained **within 14 days before** leukapheresis:

- Blood sample to assess suitability for leukapheresis/venepuncture, defined as:
  - Negative for HIV, HTLV 1, HTLV 2, syphilis and hepatitis B.
  - Minimum T-lymphocyte count of $0.25x10^9$/L.
- Confirmation the patient has relapsed or refractory neuroblastoma.
- Confirmation of GD2 expression in a tumour biopsy taken after relapse/progression, if available. Alternatively, confirmatory GD2 assessment may be carried out on optional pre-treatment biopsies or bone marrow (in those with bone marrow involvement) (see Section 7.1.2.2).
- Demographic details.
- Medical history including prior diagnosis, prior treatment, concomitant conditions/diseases and baseline signs and symptoms, concomitant treatment. *Note that, to undergo leukapheresis/venepuncture for the trial, patients must have evidence of active disease even if they do not currently require active treatment.*
- GFR (e.g. serum creatinine).
- Karnofsky performance score (if ≥16 years old, see Appendix 4) or Lansky performance score (if <16 years old, see Appendix 3).
- Female patients able to have children must have a negative result on a human chorionic gonadotropin (HCG) pregnancy test (serum or urine test is acceptable). This test should be repeated if clinically indicated **within 7 days** prior to commencement of lymphodepletion (Dose Level 2 onwards) or T-cell infusion (Dose Level 1).

Adverse events and concomitant medications: All patients should be assessed for AEs at every visit from the time of written informed consent for leukapheresis/venepuncture by the Investigator, Research Nurse or suitably qualified member of the Investigator's team. All AEs must be graded according to NCI CTCAE Version 4.02 or trial specific grading scheme for CRS (Appendix 1). Any concomitant treatment must also be recorded in the medical records and eCRF (see Section 9 for further details regarding AE reporting requirements).

Note: The leukapheresis/venepuncture may be conducted ahead of planned entry into the trial and the transduced T-cells will be frozen until required (in accordance with the approved shelf-life).

7.1.2.2 Prior to Planned 1RG-CART Infusion (Day -21 to Day -1 for Dose Level 1) or Commencement of Lymphodepletion (Day -25 to Day -5 for Dose Level 2 or Day -29 to Day -9 for Dose Levels 3 to 5)

The following baseline investigations should be carried out after written informed consent to the main trial† and within 3 weeks prior to 1RG-CART infusion for Dose Level 1, or prior to commencement of the lymphodepleting regimen for Dose Level 2 onwards.

†If more than 28 days have passed between informed consent and the start of dosing (with whichever IMP [1RG-CART/fludarabine/cyclophosphamide] is given first), then the Investigator should consider whether repeat consent should be obtained from the patient and/or patient's parent or guardian.

- Disease assessments may be performed up to 3 weeks* prior to 1RG-CART infusion for Dose Level 1, or up to 3 weeks* prior to commencement of lymphodepleting regimen for Dose Level 2 onwards. These will include at a minimum:

    - Cross sectional imaging (magnetic resonance imaging [MRI] or computerised tomography [CT]) and ultrasound.

    - [^{123}I]MIBG scintigraphy SPECT-CT. For patients with [^{123}I]MIBG non-avid tumours, consider use of [^{86}Ga]dotatate PET-CT, [^{18}F]FDG-PET-CT or technetium-99 (^{99m}Tc) bone scan. (Please see 'List of abbreviations and definition of terms' for explanation of abbreviations for imaging techniques.)

    - Bone marrow aspirate and trephine biopsy (mandatory for all patients); samples taken at the time of leukapheresis/venepuncture and falling outside of the 3 week window, do not need to be repeated (unless clinically indicated). [redacted]

  *Disease assessments performed as part of the patient's routine care prior to the patient's provision of informed consent may be used for the trial if they were performed within the 4 weeks prior to 1RG-CART infusion for Dose Level 1, or commencement of lymphodepleting regimen for Dose Level 2 onwards.

- Urine sample for assessment of catecholamines (homovallinic acid [HVA] and vanillylmandelic acid [VMA] to creatinine ratios).

- Complete physical examination (including neurological, ophthalmological and dermatological examination).

- For patients aged ≤18 years: weight and body surface area (BSA) using Children's Cancer and Leukaemia Group (CCLG) standard tables in Appendix 10. For patients aged >18 years (or patients aged ≥13 years who are obese): weight, height and BSA (calculated using the Dubois & Dubois formula).

- Blood samples for laboratory tests (**on the day prior to T-cell infusion for Dose Level 1 or within 3 days prior to commencement of lymphodepleting regimen for Dose Levels 2 and above**):
    - Haematology - haemoglobin (Hb), white blood cells (WBC) with a two point differential count (neutrophils and lymphocytes) and platelets.

    - Coagulation profile - activated partial thromboplastin time (aPTT), prothrombin time (PT), thrombin time (TT) and fibrinogen.

    - Biochemistry – sodium, potassium, adjusted calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), creatine kinase, amylase and lipase.

- Urinalysis by stick test: glucose, protein and blood.

- Tumour biopsy (optional and only in patients with amenable extramedullary sites of disease). To be performed at the same time as the bone marrow biopsy if considered to be of insignificant additional risk and there is a high probability of obtaining representative tissues (samples taken at the time of leukapheresis/venepuncture and falling outside of the 3 week window, do not need to be repeated). Several needle cores of tumour should be biopsied in accordance with normal institutional diagnostic procedures.

The following assessments should only to be reassessed if previous assessment was before Day -21 for Dose Level 1, or Day -25 for Dose Level 2, or Day -29 for Dose Levels 3 to 5:

- Estimated GFR.
- Karnofsky performance score (if ≥16 years old, see Appendix 4) or Lansky performance score (if <16 years old, see Appendix 3).

Adverse events and concomitant treatments: All patients should be assessed for AEs at every visit by the Investigator, Research Nurse or suitably qualified member of the Investigator's team. The start and stop dates of the AE must be recorded in the medical records. All AEs must be graded according to NCI CTCAE Version 4.02 or trial specific grading scheme for CRS (Appendix 1). Any concomitant treatment must also be recorded in the medical records and eCRF (see Section 9 for further details regarding AE reporting requirements).

7.2 Evaluations During Lymphodepleting Regimen (Day -4 to Day -1 for Dose Level 2 or Day -8 to Day -4 for Dose Levels 3 to 5)

- **Physical examination:** When clinically indicated, a symptom-directed physical examination (including neurological, ophthalmological and dermatological examination as clinically indicated) will be performed before administration of chemotherapy on each day (Day -4, Day -3, Day -2 and Day -1 for Dose Level 2; Day -8, Day -7, Day -6, Day -5 and Day -4 for Dose Level 3 onwards).
- **Vital signs:** Temperature, pulse rate and blood pressure (BP) will be measured before administration of chemotherapy on each day.
- **Adverse events and concomitant treatments:** Before each chemotherapy administration, an assessment of any AE experienced since the previous visit must be made. The start and stop dates of the AE together with the relationship of the event to the chemotherapy agent(s) must be recorded in the medical records. All AEs must be graded according to NCI CTCAE Version 4.02 or Appendix 1 for CRS. Any concomitant medications must also be recorded in the medical records and eCRF (see Section 9 for further details regarding AE reporting requirements).

Please also refer to the tabulated Schedule of Assessments in Section 7.9.2.

7.3 Evaluations on Day 0 (1RG-CART Administration)

- **Physical examination:** When clinically indicated, a symptom-directed physical examination (including neurological, ophthalmological and dermatological examination as clinically indicated) will be performed before 1RG-CART administration.
- **Vital signs:** Temperature, pulse rate and BP will be measured prior to 1RG-CART administration and thereafter as clinically indicated but at least hourly until 12 hours post infusion.
- **Pain score (see Appendix 9):** To be assessed prior to 1RG-CART administration.
- **Adverse events and concomitant treatments:** To be assessed prior to 1RG-CART administration as detailed in Section 7.2.
- **Blood sample for quantification of 1RG-CART:** before the 1RG-CART administration and 4 hours post infusion.
- **Blood sample for measurement of HACARA:** before the 1RG-CART administration.
- **Blood sample for safety monitoring (cytokines):** before the 1RG-CART administration and 4 hours post infusion:
    - 2 mL serum blood sample for cytokines (including but not limited to TNF-α, IFN-γ and IL-6). Serum may be stored or run daily depending on the patient's symptoms. In the event of fever or suspected cytokine storm the cytokine profile will be determined

immediately on a fresh serum sample and additional measurements will be taken as clinically indicated.

- CRP.
- LDH.
- Urate.

***Note:** the timing of immune stimulation/cytokine release is not known and may occur after Day 14 (or before Day 1). If so, assessments of cytokine release, and intensive monitoring of vital signs will start then.

Please also refer to the tabulated Schedule of Assessments in Section 7.9.2.

7.4 Evaluations from Day 1 to Day 14 (Daily Assessments) & Day 18†

Patients will be in-patients for the first 14 days after 1RG-CART infusion or resident within the vicinity of the hospital. During this time the patient will be monitored closely, at least daily. If a patient is given a split dose of 1RG-CART, the second part will be administered on Day 1 and all visits will be timed from administration of the first part of the dose on Day 0. The following minimum assessments will be performed (additional assessments should be performed as clinically indicated):

- **Physical examination:** a symptom-directed physical examination (including neurological, ophthalmological and dermatological examination as clinically indicated) daily until Day 14. If the 1RG-CART dose is being split over two days, then the physical examination on Day 1 should be carried out before the second part of the 1RG-CART dose is given.

- **Vital signs*:** Temperature, BP and pulse rate 24 hours after 1RG-CART administration (Day 1) and then daily until Day 14. If the 1RG-CART dose is being split over two days the:

  - Day 1 vital signs should be taken before 1RG-CART administration (this will be considered to be the 24 hour post Day 0 administration measurement) and thereafter as clinically indicated but at least hourly until 12 hours post infusion.

  - Day 2 vital signs should be taken 24 hours after 1RG-CART administration on Day 1.

- **Pain score (see Appendix 9):** daily until Day 14. Thereafter as clinically indicated. If the 1RG-CART dose is being split over two days, then the pain score on Day 1 should be assessed before the second part of the 1RG-CART dose is given.

- **Blood samples for haematology and biochemistry (see Section 7.1.2.2):** 24 hours after 1RG-CART administration (Day 1) and then daily until Day 14. At the Investigator's discretion daily bloods may be omitted on two occasions during this period (but not on consecutive days) in patients who are clinically well and whose blood results are stable.

- **Urinalysis by stick test:** daily until Day 14. If the 1RG-CART dose is being split over two days, then the urinalysis on Day 1 should be carried out before the second part of the 1RG-CART dose is given.

- **Blood samples for coagulation profile (see Section 7.1.2.2):** on Days 3, 7, 10 and 14.

- **Blood samples for safety monitoring (cytokines)*:** to be taken on Days 1, 2 and 3, then at least on Days 5, 7, 10, 14 and 18† or more frequently if clinically indicated (e.g. at the onset of fever or other clinical symptom suggesting possible immune activation/cytokine release):

  - 2 mL serum blood sample for cytokines (including but not limited to TNF-α, IFN-γ and IL-6). Serum may be stored or run daily depending on the patient's symptoms. In the event of

fever or suspected cytokine storm the cytokine profile will be determined immediately on a fresh serum sample and additional measurements will be taken as clinically indicated.

- CRP.
- LDH.
- Urate.

***Note:** the timing of immune stimulation/cytokine release is not known and may occur after Day 14 (or before Day 1). If so, scheduling for assessments of cytokine release, and intensive monitoring of vital signs will be appropriately adjusted.

- **Blood sample for quantification of 1RG-CART:** at 24 hours post 1RG-CART administration (Day 1), then Days 2, 3, 5, 7, 10 and 14. If the 1RG-CART dose is being split over two days, then the sample on Day 1 should be taken before the second part of the 1RG-CART dose is given.
- [redacted]
- **Urine sample:** for assessment of catecholamines on Day 14 only.
- **Adverse events and concomitant treatments:** All patients should be assessed for AEs at every visit. The start and stop dates of the AE together with the relationship of the event to the chemotherapy agent(s) and / or 1RG-CART infusion (and rituximab where administered) must be recorded in the medical records. All AEs must be graded according to NCI CTCAE Version 4.02 or trial specific grading scheme for CRS (Appendix 1). Any concomitant medications must also be recorded in the medical records and eCRF. Cytokine release syndrome (CRS) events Grade 3 to 5 should be reported as SAEs (see Section 9.2.2). Individual components of CRS (e.g. hypotension, rash, fever) do not need to be reported as SAEs, unless there is uncertainty about whether they are part of the CRS, but should be recorded as AEs in the eCRF and reported as being part of CRS. Significant sequelae of CRS (e.g. renal failure, cardiac failure, sepsis) should be reported as separate SAEs (see Section 9.2.2).

[redacted]

Please also refer to the tabulated Schedule of Assessments in Section 7.9.2.

7.5 Evaluations on Days 21, 28, 35 and Day 42† ('End of Treatment Assessment')

Patients will be monitored on Day 21 and then weekly until the 'end of treatment visit' on Day 42. The following minimum assessments will be performed at each visit (the patient may remain an in-patient and additional assessments may be performed as clinically indicated):

- **Physical examination:** a symptom-directed physical examination (including neurological, ophthalmological and dermatological examination as clinically indicated).
- **Vital signs*:** Temperature, BP and pulse rate.
- **Pain score (see Appendix 9):** If clinically indicated.
- **Blood samples for haematology and biochemistry (see Section 7.1.2.2).**
- **Urinalysis by stick test:** as clinically indicated.
- **Blood samples for clotting (see Section 7.1.2.2).**

- **Blood samples for safety monitoring (cytokines)*:** to be taken at least on Day 21 or more frequently if clinically indicated (e.g. at the onset of fever or other clinical symptom suggesting possible immune activation/cytokine release):
    - 2 mL serum blood sample for cytokines (including but not limited to TNF-α, IFN-γ and IL-6). Serum may be stored or run daily depending on the patient's symptoms. In the event of fever or suspected cytokine storm the cytokine profile will be determined immediately on a fresh serum sample and additional measurements will be taken as clinically indicated.
    - CRP.
    - LDH.
    - Urate.

  ***Note:** the timing of immune stimulation/cytokine release is not known and may occur after Day 21. If so, assessments of cytokine release, and intensive monitoring of vital signs will start then.

- **Blood sample for quantification of 1RG-CART.**
- [redacted]
- **Urine sample:** for assessment of catecholamines on Days 28 and 42 only.
- **Disease assessment:** Bone marrow aspirate and trephine biopsy (mandatory for all patients), tumour dimensions (assessed using the same methods as at baseline) on Day 28 (±6 days) only. Samples of bone marrow may be stored and used for future exploratory assessment of MRD.
- [redacted]
- [redacted]
- **Adverse events and concomitant treatments**: as detailed in Section 7.4.

†Patients who commence the lymphodepleting regimen but do not go on to receive the 1RG-CART should be followed up until Day 42 ('end of treatment visit') or until resolution of any reversible toxicities; no follow-up visits are required thereafter.

Please also refer to the tabulated Schedule of Assessments in Section 7.9.3.

7.6 Follow-Up Visits† (2 months to 2 years from Day 0)

Follow-up visits will be performed at the following time points from administration of the 1RG-CART (Day 0): 2, 3, 4, 5 and 6 months, then 9, 12 and 24 months. The following assessments will be performed as indicated:

- **Physical examination:** a symptom-directed physical examination including Karnofsky performance score (if ≥16 years old, see Appendix 4) or Lansky performance score (if <16 years old, see Appendix 3), and **weight** at each visit and including neurological, ophthalmological and dermatological examination as clinically indicated.
- **Blood sample for quantification of 1RG-CART**: at 2, 3, 4, 5, 6, 12 and 24 months.
- [redacted]

- [redacted]

- **Blood samples for haematology and biochemistry (reduced set):** at every visit.
    - Haematology – Hb, WBC with a two point differential count (neutrophils and lymphocytes), platelets
    - Biochemistry – sodium, potassium, urea, creatinine, bilirubin, ALP, ALT.

- **Disease assessment – imaging (using the same methods as at baseline):** at 2 months, 4 months, 12 and 24 months (or as clinically indicated). For patients who undergo surgical excision of residual disease, a response assessment should be documented in the eCRF before and after surgery.

- **Adverse events:** assessment of any new or ongoing AEs considered possibly related to chemotherapy, 1RG-CART or rituximab (if given) will be performed at each visit. For patients with no evidence of residual circulating transduced T-cells who start a new anticancer therapy, collection of new AEs may cease. However, SAEs considered possibly related to trial therapy are always reportable regardless of the time elapsed.

- **Concomitant medications:** assessment of concomitant treatments related to the AEs (see above). Assessment of concomitant medications may cease if collection of AEs stops (see above). Details of any new anti-cancer treatment started since the previous visit should be recorded.

Note: the timing of immune stimulation/cytokine release is not known and may occur after the Day 42 ('end of treatment visit'). If so, assessments of cytokine release and intensive monitoring of vital signs (as described in Sections 7.4 and 7.5) will start then. In the event of fever or suspected cytokine storm the cytokine profile will be determined immediately on a fresh serum sample and additional measurements will be taken as clinically indicated.

†Patients who commence the lymphodepleting regimen but do not go on to receive the 1RG-CART should be followed up until Day 42 ('end of treatment visit') or until resolution of any reversible toxicities; no follow-up visits are required thereafter.

Please also refer to the tabulated Schedule of Assessments in Section 7.9.3.

7.7 Follow-Up Visits (>2 years until the End of Trial)

Follow-up visits will be performed annually (from administration of the 1RG-CART on Day 0).

The following assessments will be performed as indicated:

- **Physical examination:** a symptom-directed physical examination including Karnofsky performance score (if ≥16 years old, see Appendix 4) or Lansky performance score (if <16 years old, see Appendix 3) and including neurological, ophthalmological and dermatological examination as clinically indicated.

- **Blood sample for quantification of 1RG-CART.**

- **Disease status:** survival status, and if patient has progressed, date of progression.

- **Adverse events:** assessment of any new or ongoing AEs considered possibly related to the 1RG-CART or rituximab (if given). For patients with no evidence of residual circulating transduced T-cells who start a new anticancer therapy, collection of new AEs may cease. However, SAEs considered possibly related to trial therapy are always reportable regardless of the time elapsed.

Please also refer to the tabulated Schedule of Assessments in Section 7.9.3.

7.8 Patients Undergoing a Second Treatment with 1RG-CART

Patients receiving a second treatment with 1RG-CART will undergo the assessments listed below and then repeat the assessments in Sections 7.2 to 7.7 according to their assigned treatment regimen. Patients having a second dose after a failed first dose must meet the criteria as detailed in Section 5.4.1.1. Patients having a second dose after a successful first dose must meet the criteria as detailed in Section 5.4.1.2. Patients having a second dose under other circumstances must meet the criteria as detailed in Section 5.4.1.3.

Within 14 days Prior to Leukapheresis/Venepuncture (only for patients in Dose Level 1 requiring a second manufacture):

- Written informed consent for leukapheresis/venepuncture (this maybe done up to 28 days before leukapheresis).
- Blood sample to assess suitability for leukapheresis/venepuncture, defined as:
    - Negative for HIV, HTLV 1, HTLV 2, syphilis and hepatitis B.
    - Minimum T-lymphocyte count of $0.25x10^9$/L.
    - No detectable circulating 1RG-CART.
- GFR (e.g. serum creatinine).
- Karnofsky performance score (if ≥16 years old, see Appendix 4) or Lansky performance score (if <16 years old, see Appendix 3).
- Female patients able to have children must have a negative result on a human chorionic gonadotropin (HCG) pregnancy test (serum or urine test is acceptable). This test should be repeated if clinically indicated **within 7 days** prior to commencement of lymphodepletion.

Within 21 days prior to planned 1RG-CART infusion (if no lymphodepletion is given) or commencement of lymphodepletion (ALL patients):

- Written informed consent (patients must sign the main study consent form again; this may be done up to 28 days before the second dose is administered).
- Complete physical examination (including neurological, ophthalmological and dermatological examination).
- For patients aged ≤18 years: weight and BSA using CCLG standard tables in Appendix 10. For patients aged >18 years (or patients aged ≥13 years who are obese): weight, height and BSA (calculated using the Dubois & Dubois formula).
- Karnofsky performance score (if ≥16 years old, see Appendix 4) or Lansky performance score (if <16 years old, see Appendix 3).
- GFR (e.g. serum creatinine).
- Female patients able to have children must have a negative result on a human chorionic gonadotropin (HCG) pregnancy test (serum or urine test is acceptable) **within 7 days** prior to planned 1RG-CART infusion (if no lymphodepletion is given) or commencement of lymphodepletion.
- Blood samples for laboratory tests:
    - Haematology – Hb, WBC with a two point differential count (neutrophils and lymphocytes) and platelets.

  - Coagulation profile - aPTT, PT, TT and fibrinogen.
  - Biochemistry – sodium, potassium, adjusted calcium, phosphate, urea, creatinine, total protein, albumin, bilirubin, ALP, ALT, creatine kinase, amylase and lipase.

- Urinalysis by stick test: glucose, protein and blood.
- Urine sample for assessment of catecholamines (homovallinic acid [HVA] and vanillylmandelic acid [VMA] to creatinine ratios), unless these have been performed in the previous 4 weeks.
- Adverse events: All patients should be assessed for AEs. All AEs must be graded according to NCI CTCAE Version 4.02 or Appendix 1 for CRS. Any concomitant medications must also be recorded in the medical records and eCRF.

Ideally all patients receiving a second dose should also undergo a **full disease assessment (including bone marrow aspirate and biopsy [optional]) to re-establish baseline measurements**, unless these have been performed in the previous 4 weeks.

7.9 Schedule of Events

7.9.1 Baseline/Pre-Trial Evaluations

	FIRST DOSE		SECOND DOSE
Observation/Investigation	**Within 14 days prior to leukapheresis (a)**	**Within 3 weeks prior to:** **- 1RG-CART infusion for Dose Level 1 (Day -21 to -1)** **- start of cyclophosphamide infusions for Dose Level 2 (Day -25 to -5)** **- start of fludarabine & cyclophosphamide infusions for Dose Levels 3 to 5 (Day -29 to -9)**	**Within 21 days prior to:** **- 1RG-CART infusion (if no lymphodepletion to be given)** **- start of lymphodepletion**
Written informed consent to undergo leukapheresis and transduce T-cells / use previous biopsy to evaluate GD2 expression (where available)	X (within 28 days)		X (within 28 days prior to leukapheresis – ONLY for patients in Dose Level 1 having a second manufacture)
Demographics	X		
Medical history (b)	X		
GFR (e.g. serum creatinine)	X		X
Karnofsky / Lansky performance status (c)	X		X
Pregnancy test (d)	X		X
Confirmation of GD2 expression	X		
Blood sample for leukapheresis eligibility (e)	X		X (within 14 days prior to leukapheresis ONLY for patients in Dose Level 1 having a second manufacture)
Adverse event evaluation	From date of informed consent for leukapheresis		
Concomitant treatments	X		
Written informed consent to main trial		X (ideally within 28 days)	X (within 28 days)
Disease assessment (f)		X	X (unless done within previous 4 weeks)
Complete physical examination		X	X
Weight (& height if >18 yrs / ≥13 yrs & obese)		X	X
Body surface area (BSA) (g)		X	X
Urine sample for assessment of catecholamines (h)		X	X (unless done within previous 4 weeks)
Urinalysis		X	X
Bloods for haematology, biochemistry & coagulation(i)		X	X
Bone marrow aspirate and trephine biopsy		X	X
Tumour biopsy (optional) (j)		X	X
Adverse event evaluation		Continually review	X

	FIRST DOSE		SECOND DOSE
Observation/Investigation	**Within 14 days prior to leukapheresis (a)**	**Within 3 weeks prior to:** **- 1RG-CART infusion for Dose Level 1 (Day -21 to -1)** **- start of cyclophosphamide infusions for Dose Level 2 (Day -25 to -5)** **- start of fludarabine & cyclophosphamide infusions for Dose Levels 3 to 5 (Day -29 to -9)**	**Within 21 days prior to:** **- 1RG-CART infusion (if no lymphodepletion to be given)** **- start of lymphodepletion**
Concomitant treatments		Continually review	X
Additional assessments (first dose only) (k):			
Estimated GFR (k)		X	
Karnofsky / Lansky performance status (c)		X	

(a) Leukapheresis/venepuncture: The leukapheresis/venepuncture may be conducted ahead of planned entry into the trial and the transduced T-cells will be frozen until required.

(b) Medical history: To include confirmation the patient has relapsed or refractory neuroblastoma. Note that, to undergo leukapheresis for the trial, patients must have evidence of active disease even if they do not currently require active treatment.

(c) Performance status: Karnofsky performance score if ≥16 years old (see Appendix 4) or Lansky performance score if <16 years old (see Appendix 3).

(d) Pregnancy test: For female patients of child bearing potential. This test (HCG serum or urine) should be repeated, if clinically indicated, within 7 days prior to commencement of the 1RG-CART infusion for Dose Level 1 or the lymphodepleting regimen for Dose Level 2 onwards.

(e) Blood sample for leukapheresis eligibility: Patients should be confirmed negative for HIV, HTLV 1, HTLV 2, syphilis and hepatitis B and have a minimum T-lymphocyte count of $0.25x10^9$/L. **For patients in Dose Level 1 receiving a second dose and requiring a second manufacture of 1RG-CART:** Patient should remain negative for HIV, HTLV1, HTLV2, syphilis and hepatitis B, have a minimum T-lymphocyte count of $0.25x10^9$/L AND have no detectable circulating 1RG-CART.

(f) Disease assessment: May be performed up to 3 weeks prior to commencement of the 1RG-CART infusion for Dose Level 1 or the lymphodepleting regimen for Dose Level 2 onwards. Disease assessments performed as part of the patient's routine care prior to the patient's provision of informed consent may be used for the trial if they were performed in the 4 weeks prior to commencement of the 1RG-CART infusion for Dose Level 1 or the lymphodepleting regimen for Dose Level 2 onwards. Disease assessment will include at a minimum: cross sectional imaging (MRI or CT) and ultrasound, [^{123}I]MIBG scintigraphy SPECT CT. For patients with [^{123}I]MIBG non-avid tumours, consider use of [^{68}Ga]dotatate PET-CT, [^{18}F]FDG-PET-CT or ^{99m}Tc bone scan.

(g) Body surface area: For patients aged ≤18 years - to be calculated using the CCLG standard tables in Appendix 10. For patients aged >18 years (or patients aged ≥13 years who are obese) – to be calculated using the Dubois & Dubois formula.

(h) Urine samples for assessment of catecholamines: HVA and VMA to creatinine ratios.

(i) Haematology and biochemistry: Samples to be taken on the day prior to commencing the 1RG-CART infusion for Dose Level 1 (Day -1) or within three days prior to commencing the lymphodepleting regimen for Dose Level 2 onwards (Day -7 to -5 for Dose Level 2, Day -11 to -9 for Dose Level 3 onwards):

- Haematology – Hb, WBC with differential count (neutrophils and lymphocytes), platelets.

- Coagulation profile - aPTT, PT, TT and fibrinogen.

- Biochemistry – sodium, potassium, adjusted calcium, phosphate, urea, creatinine, total protein albumin, bilirubin, ALP, ALT, creatine kinase, amylase and lipase.

(j) Tumour biopsy: This is optional but should be performed wherever possible in patients with amenable extramedullary sites of disease. A biopsy should only be performed at the same time as the bone marrow biopsy if considered to be of insignificant additional risk and a high probability of obtaining representative tissues. Several needle cores of tumour should be biopsied in accordance with normal institutional diagnostic procedures. Confirmation of GD2 expression in the optional tumour biopsy or bone marrow sample (if involved with neuroblastoma) are alternatives to confirmation in a specimen taken at relapse/progression.

(k) Estimated GFR & Performance status: Only to be reassessed if previous assessments were more than three weeks prior to commencing the 1RG-CART infusion for Dose Level 1 (i.e. before Day -21) or the lymphodepleting regimen for Dose Level 2 onwards (i.e. before Day -25 for Dose Level 2, Day -29 for Dose Levels 3 to 5).

7.9.2 Evaluations During Treatment Phase and up to Day 18*

Observation/ Investigation	Treatment phase▲						Intensive monitoring													
	Day -8	Day -4/-7	Day -3/-6	Day -2/-5	Day -1/-4	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 18†
Physical examination (l)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Vital signs (temperature, BP and pulse rate) (m)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Pain score (n)						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Adverse event evaluation (o)	Continually review						Continually review														
Concomitant treatments	Continually review						Continually review														
[redacted]																					
Bloods for cytokines (p)						X	X	X	X		X		X			X				X	X
Bloods for C-reactive protein (CRP) (p)						X	X	X	X		X		X			X				X	X
Bloods for LDH and urate (p)						X	X	X	X		X		X			X				X	X
Bloods for quantification of 1RG-CART (q)						X	X	X	X		X		X			X				X	
[redacted]																					
Bloods for haematology and biochemistry							X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Bloods for coagulation profile									X				X			X				X	
Urinalysis							X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine sample for assessment of catecholamines																				X	
Cyclophosphamide administration (s)		X	X	X	X																
Fludarabine administration (s)	X	X	X	X	X																
1RG-CART administration (s)						X	(X)														

†Day 18 visit is intended to take place when levels of 1RG-CART in the blood peak and therefore the exact timing of this visit may change based on emerging PD data.

▲Treatment phase: Conditioning regimen to be administered on Days -4, -3, -2 and -1 for Dose Level 2 & on Days -8, -7, -6, -5 and -4 for Dose Level 3 onwards.

(l) Physical examination: a symptom directed physical examination (including neurological, ophthalmological and dermatological examination as clinically indicated) to be performed as clinically indicated prior to each administration of chemotherapy, i.e. on Days -4 to -1 for Dose Level 2, Days -8 to -4 for Dose Level 3 onwards. Then for all cohorts: prior to administration of 1RG-CART on Day 0, and daily until Day 14. If 1RG-CART is being given as a split dose, the physical examination on Day 1 should be performed prior to the second administration of 1RG-CART.

(m) Vital signs: Temperature, pulse rate and blood pressure (BP) will be performed prior to each administration of chemotherapy, i.e. on Days -4 to -1 for Dose Level 2, Days -8 to -4 for Dose Level 3 onwards. Then for all cohorts: prior to administration of 1RG-CART on Day 0 and then at least hourly (or more frequently if clinically indicated) until 12 hours post dose, then daily until Day 14. If the 1RG-CART is being given as a split dose: Day 1 vital signs should be taken before the second 1RG-CART administration and thereafter as clinically indicated but at least hourly until 12 hours post infusion. Day 2 vital signs should then be taken 24 hours after 1RG-CART administration on Day 1. *The timing of immune stimulation / cytokine release is not known, in the event of symptoms suggestive of cytokine release syndrome intensive vital signs monitoring will commence and continue as clinically indicated.*

(n) Pain score: to be performed prior to administration of 1RG-CART on Day 0 and daily until Day 14 (thereafter as clinically indicated). See Appendix 9. If 1RG-CART is being given as a split dose, the pain score on Day 1 should be performed prior to the second administration of 1RG-CART.

(o) Adverse events: Cytokine release syndrome (CRS) events Grade 3 to 5 should be reported as SAEs (see Section 9.2.2). Individual components of CRS (e.g. hypotension, rash, fever) do not need to be reported as SAEs unless there is uncertainty about whether they are part of CRS, but should be recorded as AEs in the eCRF and reported as being part of CRS. Significant sequelae of CRS (e.g. renal failure, cardiac failure, sepsis) should be reported as separate SAEs (see Section 9.2.2).

(p) Bloods for safety monitoring (cytokines, CRP, LDH and urate) will be taken at least on Day 0 (prior to 1RG-CART infusion and 4 hours post infusion), 1, 2, 3, 5, 7, 10, 14 and 18†. *The timing of immune stimulation / cytokine release is not known, in the event of symptoms suggestive of cytokine release syndrome blood samples for assessment of cytokines, CRP, LDH and urate will be taken as clinically indicated.*

(q) Bloods for quantification of 1RG-CART: To be performed on Day 0 prior to 1RG-CART and 4 hours post infusion, then 24 hours post infusion (Day 1), and on Days 2 (±12 h), 3 (±12 h), 5, 7, 10 and 14 (±1 day). If 1RG-CART is being given as a split dose, the sample on Day 1 should be taken prior to the second administration of 1RG-CART.

[redacted]

(s) ATIMP/IMPs to be given as follows (dosing for expansion cohort to be defined during the dose escalation part of the trial):

- Cyclophosphamide: To be given on Days -4 to -1 for Dose Level 2 and Days -8 to -4 for Dose Level 3 onwards.

- Fludarabine: To be given on Days -8 to -4 for Dose Level 3 onwards. (Fludarabine to be given first on days when given with cyclophosphamide.)

- 1RG-CART: To be given on Day 0 for all cohorts. In order to administer the higher doses of 1RG-CART in Dose Level 5, it may be necessary to split the dose over two consecutive days (Day 0 and Day 1).

***Patients receiving a second dose of 1RG-CART will repeat the assessments tabulated in Section 7.9.2 (and described in Sections 7.2 to 7.7) according to the treatment regimen assigned.**

7.9.3 Evaluations During Follow-Up (Day 21 to End of the Trial)

Observation/ Investigation	**Weekly monitoring**				**Follow-up** *(only required for patients who have received 1RG-CART)*								
	Day 21	Day 28	Day 35	Day 42	2 months	3 months	4 months	5 months	6 months	9 months	12 months	24 months	Annually from Day 0 (until End of Trial)
Physical examination (t)	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs (temperature, BP and pulse rate) (u)	X	X	X	X									
Karnofsky / Lansky performance status					X	X	X	X	X	X	X	X	X
Weight					X	X	X	X	X	X	X	X	
Adverse event evaluation	Continually review				Continually review (v)								
Concomitant treatments	Continually review				Continually review (v)								
[redacted]													
Bloods for cytokines (w)	X												
Bloods for C-reactive protein (CRP) (w)	X												
Bloods for LDH and urate (w)	X												
Bloods for quantification of 1RG-CART (x)	X	X	X	X	X	X	X	X	X		X	X	X
[redacted]													
Bloods for haematology and biochemistry (y)	X	X	X	X	X	X	X	X	X	X	X	X	
Bloods for coagulation profile	X	X	X	X									
Urinalysis (as clinically indicated)	X	X	X	X									
Urine sample for assessment of catecholamines		X		X									
Disease assessment (imaging) (z)		X (±6 days)			X		X				X	X	
Bone marrow aspirate and trephine (z)		X (±6 days)			X		X						
[redacted]													
progression)													

(t) Physical examination: a symptom directed physical examination (including neurological, ophthalmological and dermatological examination as clinically indicated) to be performed on Days 21, 28, 35 and 42, then at 2, 3, 4, 5, 6, 9, 12 and 24 months and then annually until the End of the Trial.

(u) Vital signs: Temperature, pulse rate and blood pressure (BP) will be performed on Days 21, 28, 35 and 42. *The timing of immune stimulation / cytokine release is not known, in the event of symptoms suggestive of cytokine release syndrome intensive vital monitoring will commence and continue as clinically indicated.*

(v) Adverse events / concomitant medications: Once the patient is in follow-up (from 2 months until the End of the Trial) only new or ongoing adverse events considered possibly, probably or definitely related to chemotherapy or 1RG-CART need to be assessed. For patients with no evidence of residual circulating transduced T-cells who start a new anti-cancer therapy, collection of new AEs may cease. However, SAEs considered related to trial therapy are always reportable regardless of the time elapsed. Only concomitant medications related to the AEs which require reporting (see above) and details of any new anti-cancer treatment (up until the 2 year follow-up visit) should be recorded.

Cytokine release syndrome (CRS) events Grade 3 to 5 should be reported as SAEs (see Section 9.2.2). Individual components of CRS (e.g. hypotension, rash, fever) do not need to be reported as SAEs unless there is uncertainty about whether they are part of CRS, but should be recorded as AEs in the eCRF and reported as being part of CRS. Significant sequelae of CRS (e.g. renal failure, cardiac failure, sepsis) should be reported as separate SAEs (see Section 9.2.2).

(w) Bloods for safety monitoring (cytokines, CRP, LDH and urate) will be taken at least on Day 21. *The timing of immune stimulation / cytokine release is not known, in the event of symptoms suggestive of cytokine release syndrome blood samples for assessment of cytokines, CRP, LDH and urate will be taken as clinically indicated.*

(x) Bloods for quantification of 1RG-CART: To be performed:

- on Days 21, 28, 35 and 42 (±2 days).
- at 2, 3, 4, 5, 6 and 12 months (±1 week).
- at 24 months (±2 weeks).
- annually until the End of the Trial (±2 weeks).

(y) Bloods for haematology and biochemistry: Only a reduced set of bloods is required at 2, 3, 4, 5, 6, 9, 12 and 24 months as follows:

Haematology – Hb, WBC with differential (neutrophils and lymphocytes), platelets.

Biochemistry – sodium, potassium, urea, creatinine, bilirubin, ALP, ALT.

(z) Bone marrow aspirate and biopsy / tumour assessment (imaging):

Bone marrow aspirate and biopsy - To be performed on Day 28 (±6 days), then at 2 and 4 months (only for patients with evidence of disease or 1RG-CART in the bone marrow on previous assessment). [redacted]

Disease assessment (imaging – using the same methods as at baseline) - To be performed on Day 28 (±6 days), then at 2, 4, 12 and 24 months from Day 0 (or as clinically indicated).

Patients who undergo surgical excision of residual disease require a response assessment eCRF page to be completed before and after surgery.

[redacted]

8 PHARMACODYNAMIC ASSESSMENTS

Please refer to the 1RG-CART Laboratory Manual for detailed instructions.

Sample collection schemes may be revised during the trial upon collection of more pharmacodynamic data.

8.1 Secondary Assessments

8.1.1 Estimation of 1RG-CART Counts in Peripheral Blood (Flow Cytometry)

Circulating transduced T-cells will be measured by flow cytometry using whole blood, according to agreed SOPs and validated methods.

5 mL of blood will be collected into ethylenediaminetetraacetic acid (EDTA) tubes from all patients at the following time points:

- Day 0 (pre-infusion and 4 hours post end of infusion)
- Day 1 (24 hours post end of infusion)
- Days 2, 3 (±12 h)
- Days 5, 7, 10, 14 (±1 day)
- Days 21, 28, 35 and 42 (±2 days)
- At 2, 3, 4, 5, 6, 12 (±1 week) and 24 months (±2 weeks)
- Annually until the End of the Trial (±2 weeks)

The approximate total volume of blood withdrawn from each patient (in the first two years of the trial) for these samples will be 100 mL.

The fluorescence activated cell sorting (FACS) assay generates data in terms of percentage of transduced cells per mL of whole blood.

Please refer to the 1RG-CART Laboratory Manual for handling and storage of samples.

8.2 Tertiary/Research Assessments

8.2.1 [redacted]

[redacted]

8.2.2 [redacted]

[redacted]

- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

8.2.3 [redacted]

[redacted]

[redacted]

[redacted]

8.2.4 [redacted]

[redacted]

[redacted]

[redacted]

8.2.5 [redacted]

[redacted]

[redacted]

- [redacted]
- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

8.3 Additional Research Assays for Safety / Eligibility / Disease Monitoring

8.3.1 Serum Cytokine Profiles (Safety Monitoring)

Serum cytokine profile (minimum dataset of TNF-α, IFN-γ, and IL-6) will be measured in 2 mL of serum using cytokine bead array (Becton Dickinson). Serum samples will be frozen for cytokine measurements as follows:

- Day 0 (pre-infusion and 4 hours post end of infusion),
- Day 1 (24 hours post end of infusion) and then
- Days 2, 3, 5, 7, 10, 14, 18 (timing of this sample may vary depending on when 1RG-CART levels in the blood peak) and 21.

Samples will be analysed as a single test set. In the event of fever or suspected cytokine storm the cytokine profile will be determined immediately on a fresh serum sample and additional measurements will be taken as clinically indicated.

The minimum approximate total volume of blood withdrawn from each patient for these samples will be 22 mL. However, in the event of fever or suspected cytokine storm additional samples will be collected as required to ensure the safety of the patient.

Please refer to the 1RG-CART Laboratory Manual for handling and storage of samples.

8.3.2 GD2 Expression in Tumour Biopsies / Bone Marrow (Eligibility & Disease Monitoring)

GD2 expression will be evaluated by immunohistochemistry performed on tissue from existing tumour samples (collected as part of routine care at the time of disease relapse/progression) and the optional tumour biopsies taken as part of the study at pre-treatment and on Day 28 (±6 days), for the estimation of 1RG-CART as detailed in Section 8.2.4.

Alternatively, GD2 expression will be assessed on tumour detected in bone marrow trephines taken as part of routine clinical assessment. GD2 expression will be evaluated by immunohistochemistry or other techniques according to agreed SOPs and methods.

9 ASSESSMENT OF SAFETY

9.1 Investigator Responsibilities

The Investigator is responsible for monitoring the safety of patients who have enrolled in the trial and for accurately documenting and reporting information as described in the following sections.

9.1.1 Medical Cover

The CI/PI is responsible for ensuring patients have access to 24 hour advice and/or care. Patients will be provided with the necessary contact numbers for both normal working and out of hours care. A copy of the protocol must be made available out of hours to ward staff and clinicians on call so that the appropriate advice may be given to the patient, the patient's relative or other care giver (for example GP). The CI/PI must ensure that should the on call clinician or ward staff require more advice than is in this protocol, that they have access to the Investigator or delegated members of the investigator's team who can answer any questions.

9.2 Adverse Event Definitions

9.2.1 Adverse Event

An AE is any untoward, undesired or unplanned occurrence in a patient administered an IMP, a comparator product or an approved drug.

An AE can be a sign, symptom, disease, and/or laboratory or physiological observation that may or may not be related to the IMP or comparator.

An AE includes but is not limited to those in the following list.

- A clinically significant worsening of a pre-existing condition. This includes conditions that may resolve completely and then become abnormal again.
- AEs occurring from an overdose of an IMP, whether accidental or intentional.
- AEs occurring from lack of efficacy of an IMP, for example, if the Investigator suspects that a drug batch is not efficacious or if the Investigator suspects that the IMP has contributed to disease progression.

Other reportable events that must be treated as AEs are listed below:

- Pregnancy exposure to the IMP. Any pregnancy occurring in a patient or a patient's partner during treatment with an IMP or occurring within six months of the last IMP administration (12 months for female patients who become pregnant after receiving cyclophosphamide or rituximab), must be reported to the Pharmacovigilance Department (PV) in the same timelines as an SAE. These should be reported even if the patient is withdrawn from the trial.
- Overdose with or without an AE (any dose above that specified in the protocol, not necessarily intentional).
- Medication error (any unintentional error in the dispensing or administration of an IMP).
- Occupational exposure / accidental exposure (to a person other than the patient, for example spilling of IMP on hands of nurse or splashing in the eye).
- Any AE that could be related to the protocol procedures, and which could modify the conduct of the trial.

Any occurrence of these events should be reported in the same manner as SAEs (see Section 9.4).

9.2.2 Serious Adverse Events

A SAE is any AE, regardless of dose, causality or expectedness, that:

- results in death;
- is life-threatening^;
- requires in-patient hospitalisation *or* prolongs existing in-patient hospitalisation (some hospitalisations are exempt from SAE reporting – e.g. hospital admissions planned prior to the patient entering the trial; overnight stays for planned procedures such a blood transfusions (Section 9.4.1);
- results in persistent or significant incapacity or disability;
- is a congenital anomaly or birth defect;
- is any other medically important event.*

For fatal SAEs, wherever possible report the cause of death as an SAE with a fatal outcome rather than reporting death as the SAE term. When available the autopsy report will be provided to the Sponsor.

^ A life-threatening event is defined as an event when the patient was at substantial risk of dying at the time of the adverse event, or use or continued use of the device or other medical product might have resulted in the death of the patient.

*A medically important event is defined as any event that may jeopardise the patient or may require intervention to prevent one of the outcomes listed above. Examples include allergic bronchospasm (a serious problem with breathing) requiring treatment in an emergency room, serious blood dyscrasias (blood disorders) or seizures/convulsions that do not result in hospitalisation. The development of drug dependence or drug abuse would also be examples of important medical events.

Trial–specific medically important events

For this trial any event which meets the definition of a DLT (as defined in Section 3.4) or requires the administration of rituximab should be reported as a SAE.

Grade 3 to 5 CRS events should be reported as medically important events, if they do not satisfy the other criteria for an SAE. Individual components (e.g. hypotension, rash, fever), should not be reported as individual SAEs, unless there is uncertainty about whether they are part of the CRS. However, significant sequelae (e.g. renal failure, cardiac failure, sepsis) should be reported as separate SAEs.

If during the course of the study, additional medically important events are identified, these will be communicated to site and the protocol will be updated to reflect this.

9.2.3 Suspected, Unexpected, Serious Adverse Reactions

A SUSAR is a suspected, unexpected, serious adverse reaction. All AEs and SAEs will be assessed by the Sponsor for seriousness, causality and expectedness. The Sponsor's PV Department will expedite all SUSARs to the relevant Competent Authority/Authorities and the relevant Ethics Committee(s) within the timelines specified in legislation (SI 2004/1031 as amended).

Determining Adverse Event Causality

The relationship of an AE to the IMPs (1RG-CART/cyclophosphamide/fludarabine) and/or NIMPs (rituximab/antihistamine) is determined as follows.

Highly probable

- Starts within a time related to the IMP administration and
- No obvious alternative medical explanation.

Probable

- Starts within a time related to the IMP administration and
- Cannot be reasonably explained by known characteristics of the patient's clinical state.

Possible

- Starts within a time related to the IMP administration and
- A causal relationship between the IMP and the AE is at least a reasonable possibility.

Unlikely

- The time association or the patient's clinical state is such that the trial drug is not likely to have had an association with the observed effect.

Not related

- The AE is definitely not associated with the IMP administered.

Note: Drug-related refers to events assessed as possible, probable or highly probable.

The Investigator must endeavour to obtain sufficient information to determine the causality of the AE (i.e. IMP, other illness, progressive malignancy etc) and must provide his/her opinion of the causal relationship between each AE and IMP. This may require instituting supplementary investigations of significant AEs based on their clinical judgement of the likely causative factors and/or include seeking a further opinion from a specialist in the field of the AE.

The following guidance should be taken in to account when assessing the causality of an AE:

- Previous experience with the IMP and whether the AE is known to have occurred with the IMP.
- Alternative explanations for the AE such as concomitant medications, concurrent illness, non-medicinal therapies, diagnostic tests, procedures or other confounding effects.
- Timing of the events between administration of the IMP and the AE.
- IMP blood levels and evidence, if any, of overdose.
- De-challenge, that is, if the IMP was discontinued or the dosage reduced, what happened to the adverse reaction?
- Re-challenge, that is, what happened if the IMP was restarted after the AE had resolved?

9.2.4 Expectedness

Assessment of expectedness for 1RG-CART will be made by the PV Department against the current version of the 1RG-CART IB. Expectedness for cyclophosphamide, fludarabine, rituximab and the antihistamine will be made against their respective SmPCs.

9.3 Collection of Safety Information

9.3.1 Screening Failures

For patients who fail screening (for leukapheresis or the main study), SAEs must be reported to the PV Department from the date of first consent until the date the patient is confirmed as ineligible.

9.3.2 Eligible Patients

For eligible patients, SAE and AE collection and monitoring will commence at the time the patient gives their written consent to undergo trial-specific leukapheresis.

For patients who receive 1RG-CART, monitoring will continue until they withdraw from the trial or the trial ends (whichever comes first). However, once patients enter the follow up phase of the study (2 months to 2 years from 1RG-CART administration) only new and ongoing AEs considered related (possible, probable or highly probable) to chemotherapy, 1RG-CART or rituximab (if given) will be collected. Once a patient has completed the 24 month visit, only AEs related to 1RG-CART (or rituximab, if given) will be collected. For patients with no evidence of residual circulating transduced T-cells who start a new anticancer therapy, collection of new AEs may cease.

For patients who commence the lymphodepleting regimen but do not go on to receive 1RG-CART, monitoring will continue until the Day 42 'end of treatment visit' or until resolution of any reversible toxicities.

Should an Investigator become aware of any drug-related SAEs after the patient has completed the protocol-mandated follow-up period (as specified above), these must also be reported to the Sponsor within the expedited timelines in Section 9.4.

9.3.3 Follow-Up of AEs and SAEs

Follow-up of AEs with a causality of possible, probable or highly probable will continue until the events resolve or stabilise.

The Sponsor's PV Department will make requests for further information on SAEs to the trial site at regular intervals. Requested follow-up information should be reported to the PV Department in a timely manner and as soon as possible after receipt of the follow-up request. For fatal or life-threatening cases, follow-up information must be reported to the PV Department as soon as possible.

9.4 Reporting of SAEs to the Sponsor's Pharmacovigilance Department

All SAEs, regardless of causality, must be reported to the PV Department in an expedited manner.

SAEs should be documented on an SAE report form, using the completion guidelines provided.

The SAE report form should be emailed to Pharmacovigilance Department within 24 hours of site staff becoming aware of the SAE.

[redacted]

Each episode of an SAE must be recorded on a separate SAE report form. The NCI CTCAE Version 4.02 (or Appendix 1 for CRS) must be used to grade each SAE, and the worst grade recorded. If new or amended information on a previously reported SAE becomes available, the Investigator should report this to the PV Department on a new SAE report form.

If the SAE has not been reported within the specified timeframes, a reason for lateness must be added to the form when sending the SAE report to the PV Department.

Should the investigator become aware of any drug-related SAEs after the patient has withdrawn from the study, these must also be reported to the PV Department within the timelines specified above.

9.4.1 Events Exempt from being Reported as SAEs to the Pharmacovigilance Department

Events specified in this section do not require reporting as SAEs in this trial, unless hospitalisation is prolonged for any reason and then an SAE form must be completed. The events must still be recorded in the appropriate section of the eCRF.

Elective admissions – Elective admissions to hospital for planned procedures or for primarily social reasons or convenience are not SAEs, and do not require SAE reporting. Hospitalisation for administration of the IMP according to the trial protocol is also exempt from being reported as an SAE. However, if during hospitalisation for IMP administration the patient experiences an adverse event that would normally qualify as an SAE, this event should still be reported as an SAE.

Death due to disease progression – cases of death due to disease progression do not require SAE reporting, unless considered related to an IMP.

9.5 Recording of Adverse Events and Serious Adverse Events in eCRFs

All AEs, including SAEs, must be recorded in the eCRF for eligible patients. This includes AEs/SAEs attributable to 1RG-CART, cyclophosphamide, fludarabine or rituximab. All concomitant medications, including herbal medications and supplements must be recorded (herbal, homeopathic agents or food supplements are not allowed between Day -10 and Day 20 unless recommended by the PI). Any therapy used to treat the event must be recorded. The eCRF will be reconciled with the safety database during and at the end of the trial. Therefore, the sites should ensure the data entered on the SAE report form and the data entered into the eCRF are consistent. The Sponsor's Medical Advisor and the Investigator(s) will regularly review the safety data from both the safety and the clinical database.

9.6 Urgent Safety Measures

The Sponsor or Investigator may take appropriate urgent safety measures (USMs) in order to protect the patient of a clinical trial against any immediate hazard to their health or safety. This includes procedures taken to protect patients from pandemics or infections that pose serious risk to human health.

USMs may be taken without prior authorisation from the competent authority.

The Medicines and Healthcare products Regulatory Agency (MHRA) and the main Research Ethics Committee (REC) must be notified within three days of such measures being taken.

Should the site initiate a USM, the Investigator must inform the Sponsor immediately either by:

- [redacted]
- [redacted]

The notification must include:

- the date of the USM;
- who took the decision; and
- why action was taken.

The Sponsor will then notify the MHRA and the REC within three days of USM initiation.

9.7 Pregnancy

The Investigator must make every effort to try and ensure that a clinical trial patient or a partner of a clinical trial patient does not become pregnant during the trial or for six months after the last IMP administration. For female patients who receive cyclophosphamide or rituximab, the contraceptive period should be extended to 12 months after cyclophosphamide/rituximab administration. Where appropriate, this should be done as part of the consent process by explaining clearly to the patient and/or their parent or guardian the potential dangers of becoming pregnant and also providing each patient with information about appropriate medically approved contraception. Where appropriate, two forms of medically approved contraception should be used, such as:

- oral contraceptives and condom;
- intra-uterine device and condom;
- diaphragms with spermicidal gel and condom.

Contraception should be effective before the patient is enrolled on the trial, throughout the trial and for six months after the last IMP administration (in female patients receiving cyclophosphamide or rituximab it should be extended to 12 months after cyclophosphamide/rituximab administration).

Where appropriate, it should be explained to the patient and/or patient's parent or guardian that if his partner is pregnant or breast-feeding when he is enrolled on the trial, the patient should use barrier method contraception (condom plus spermicidal gel) to prevent the unborn baby or the baby being exposed to the trial treatment.

However, if a patient or a partner of a patient does become pregnant, the reporting procedures below must be followed.

Any pregnancy occurring in a patient or a patient's partner during treatment with an IMP or occurring within six months of last IMP administration (or 12 months from last administration of cyclophosphamide or rituximab for female patients) must be reported to the PV Department within 24 hours of the site staff becoming aware of it using a Pregnancy Form. It is the Investigator's responsibility to obtain consent for follow-up from the patient or patient's partner and/or their parent or guardian. In addition, the Investigator must be made aware of the need to obtain contact details for the patient's partner's General Practitioner. The PV Department will follow-up all pregnancies for the pregnancy outcome via the Investigator, using a Pregnancy Form.

The Investigator must ensure that all patients and/or the patient's parent or guardian are aware at the start of a clinical trial of the importance of reporting all pregnancies (in themselves and their partners) that occur whilst being treated with the IMP and occurring up to six months after the last IMP administration (or 12 months from last administration of cyclophosphamide or rituximab for female patients). The Investigator should offer counselling to the patient and/or the partner, and discuss the risks of continuing with the pregnancy and the possible effects on the foetus. Monitoring of the patient or partner should continue until the conclusion of the pregnancy, if the patient or patient's partner (or their parent or guardian) has consented to this. Monitoring of the baby should continue until 12 months after birth, if the patient or patient's partner (or their parent or guardian) has consented to this.

10 ASSESSMENT OF EFFICACY

In this trial, response will be assessed using RECIST 1.1 criteria ([53]; see Appendix 6), immune-related Response Criteria (irRC) ([54], 2009; see Appendix 7), and International Neuroblastoma Response Criteria (INRC) ([55]; see Appendix 8).In order to satisfy the requirements of all three response criteria, please follow the guidelines outlined in the sections below and in Appendices 6, 7 and 8.

10.1 Measurement of Disease

Key features of the three sets of response criteria (as published) are summarised in the table below.

	RECIST 1.1	**irRC**	**INRC**
Key reference	[53]	[54]	[55]
Types of lesions identified at baseline	Target & non-target	Index and non-index	Primary and metastases
Measurements of measurable lesions	One dimension (longest diameter)	Two dimensions (longest perpendicular diameters)	Three dimensions (volume, not otherwise specified)
Number of lesions measured at baseline	Maximum of 5 (maximum of 2 lesions per organ)	5 lesions per organ, up to 10 visceral lesions total and 5 cutaneous lesions	Not specified
Minimum size of measurable lesion at baseline	10 mm (15 mm for lymph nodes, 20 mm for CXR)	Not specified (but presumed to be ≥5x5 mm)	Not specified
New lesions	Considered progressive disease	If measurable (≥5x5 mm) added to tumour burden; up to 5 new lesions per organ; 5 new cutaneous lesions ,and 10 visceral lesions	Considered progressive disease
Response categories	CR, PR, SD and PD	CR, PR, SD and PD	CR, VGPR, PR, MR, NR and PD
Key differences in PR criteria:	Requires 30% reduction in sum of diameters of target lesions, confirmed ≥4 weeks later	Requires decrease in tumour burden ≥50% relative to baseline, confirmed ≥4 weeks later	Two categories of PR based on separate measurements of primary tumour and individual metastases
Key differences in SD criteria:	Neither PR nor PD by RECIST 1.1	Neither PR nor PD by irRC	Divided into MR and NR; some MRs would be PR by RECIST 1.1 or irRC
Key differences in PD criteria	Requires a 20% increase in the sum of diameters of target lesions or any new lesion (no confirmation required)	Requires increase in tumour burden ≥25% relative to nadir, confirmed ≥4 weeks later	Requires an increase in any measurable lesion by >25% or any new lesion or a positive bone marrow after a previous negative marrow. Progression within 4 months = PD regardless of interim PR/CR and no confirmation required
Other differences in response criteria	Bone marrow and catecholamines not specifically covered	Bone marrow and catecholamines not specifically covered	Bone marrow and catecholamines specifically included in response criteria

Abbreviations: CR=complete response; CXR= chest x-ray; MR=mixed response; NR= no response; PD=progressive disease; PR=partial response; SD=stable disease; VGPR= very good partial response

In this trial, in order to simplify data collection and verification, and to facilitate data analysis:

- Bidimensional lesion measurements will be collected for all lesions that are bidimensionally measurable according to irRC criteria at baseline (i.e. ≥5x5 mm). Measurements will be collected for a maximum of 5 lesions per organ, including up to 10 visceral lesions in total, and 5 cutaneous lesions (index lesions).
- Bidimensional lesion measurements will be collected for all measurable new lesions (i.e. those measuring i.e. ≥5x5 mm).
- Bidimensional lesion measurements (including baseline/index and new lesion measurements) will be used to calculate response and progression according to irRC criteria.
- Bidimensional lesion measurements of baseline/index lesions will be used to calculate response and progression according to INRC criteria.
- Target lesions will be selected from the index lesions based on RECIST 1.1 criteria. A maximum of 5 lesions (2 per organ) will be selected based on the longest diameter measured and other RECIST 1.1 criteria (see Appendix 6).
- Target lesion diameters will be used to assess response according to RECIST 1.1 criteria.
- Bone marrow and catecholamine data will be used in the assessment of CR, PR and PD according to INRC, and bone marrow and catecholamine data will be used in the assessment of CR according to RECIST 1.1 and irRC.
- Whenever possible, patients with progressive disease (by any criteria) should have a confirmatory assessment at least 4 weeks later.
- Patients with a PR or CR should have a confirmatory assessment at least 4 weeks later.

10.2 Timing and Type of Tumour Assessments

A thorough clinical and radiological evaluation of malignancy, as judged appropriate by the Investigator, and in line with the protocol, must be performed before a patient receives their first dose of 1RG-CART or lymphodepleting regimen (whichever comes first).

Whenever possible, the same methods of lesion assessment at baseline must be used to follow these lesions throughout the trial. To ensure compatibility, the radiological assessments used to assess response must be performed using identical techniques. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the anti-tumour effect of a treatment.

All baseline radiological assessments must be performed within **three** weeks before receiving 1RG-CART or lymphodepleting regimen (whichever comes first), unless they were performed as part of the patient's routine care, in which case they may be performed within **four** weeks before commencing treatment.

All clinical measurements to assess response must be performed within **one** week before the patient's first dose of 1RG-CART or lymphodepleting regimen (whichever comes first).

Copies of the scans must be available for external independent review if requested by the Sponsor.

10.2.1 Baseline Evaluations

These must include radiological measurements and as indicated chest computerised tomography (CT) scan or magnetic resonance imaging (MRI), abdominal CT scan or MRI, bone scan and/or clinical measurements as appropriate. All areas of disease present must be documented (even if specific lesions are not going to be followed for response) and the measurements of all measurable lesions must be recorded clearly on the scan reports. Any non-measurable lesions must be stated as being present. For clinical measurements, documentation by colour photography including a ruler to estimate the size of the lesion is strongly recommended, as this aids external independent review of responses.

10.2.2 Evaluations During and at 'Off-Study'

Tumour assessments must be repeated on Day 28 (±6 days) and at 2, 4, 12 and 24 months post 1RG-CART administration or more frequently, when clinically indicated. All lesions measured at baseline must be measured at every subsequent disease assessment, and recorded clearly on the scan reports. All non-measurable lesions noted at baseline must be noted on the scan report as present or absent.

All patients, who are removed from the trial for reasons other than progressive disease, must be re-evaluated at the time of treatment discontinuation, unless a tumour assessment were performed within the previous four weeks.

It is the responsibility of the PI to ensure that the radiologists are aware of the requirement to follow-up and measure every measurable/index/target lesion mentioned at baseline and comment on the other lesions in accordance with RECIST 1.1, irRC and INRC criteria.

10.3 Tumour Response

All patients who receive at least one dose of 1RG-CART and who have a baseline and at least one post-treatment assessment of disease will be evaluable for response. Patients who experience clear disease progression without a formal post-treatment disease assessment will be considered non-responders. To be assigned a status of stable disease (SD), follow-up measurements must have met the SD criteria at least once and at least six weeks after the initial dose of the 1RG-CART is given.

Tumour response should be classified as "not evaluable" (NE), only when it is not possible to classify it under another response category, for example, when baseline and/or follow-up assessment is not performed or not performed appropriately.

Expert reviewers appointed by the Sponsor may undertake an independent review of the Investigator's assessed objective responses (CR and PR). The expert reviewers will include at least one specialist who is not an Investigator in the trial. In case of disagreement between the Investigator's and the expert reviewers' assessment, the Sponsor's assessment will be used to make any decisions with regard to recruitment etc. The independent reviewer's assessment will also be documented in the final clinical study report (CSR) along with the original assessment made by the Investigator. The eCRF will reflect the Investigator's opinion.

10.3.1 Recording of Response in the eCRF

The applicable overall response category (for RECIST, irRC and INRC) for each visit that includes disease assessment must be recorded in the eCRF. The overall response category for RECIST (see Appendix 6), irRC (see Appendix 7) and INRC (see Appendix 8) should also be documented in the eCRF.

10.4 Overall Survival and Progression-Free Survival

10.4.1 Overall Survival

All patients who receive 1RG-CART will be evaluable for overall survival, regardless of whether they subsequently receive other anti-cancer treatments. Overall survival will be measured from the date of administration of the first dose of 1RG-CART. Since only limited follow-up (until Day 42) is planned for patients who receive the lymphodepleting regimen only, these patients will not be included in analyses of overall survival unless the patient's death is considered primarily due to the lymphodepleting regimen.

10.4.2 Progression-Free Survival

All patients who receive 1RG-CART and who complete a baseline and at least one post-treatment disease assessment (or experience clear disease progression without a formal post-treatment disease

assessment) will be evaluable for progression-free survival, regardless of whether they subsequently receive other anti-cancer treatments. This will be assessed according to all three tumour response criteria (RECIST 1.1, irRC and INRC). Progression-free survival will be measured from the date of administration of the first dose of 1RG-CART. Since only limited follow-up (until Day 42) is planned for patients who receive the lymphodepleting regimen only, these patients will not be included in analyses of progression-free survival unless there is clear evidence of disease progression in the 42-day follow-up period or the patient's death is considered primarily due to the lymphodepleting regimen.

11 PATIENT WITHDRAWAL BEFORE THE END OF THE TRIAL

Due to the possibility that the 1RG-CART may persist for many years, patients who are given the transduced T-cells should remain on the trial until the End of Trial is declared, at which time they will be re-enrolled and consented to a follow-on protocol (see Section 12). The Investigator must make every reasonable effort to keep each patient given 1RG-CART on the trial in order to collect long-term safety data. The main justifiable reasons for a patient to be withdrawn from the trial after administration of 1RG-CART are:

- Withdrawal of consent;
- Loss to follow-up;

However, patients with progressive disease necessitating alternative anti-cancer treatment may be withdrawn from the trial (and long-term follow-up) if there are no detectable transduced T-cells in the peripheral blood and maintaining follow-up is difficult.

Patients who commence the lymphodepleting regimen but do not go on to receive the 1RG-CART should be assessed as clinically indicated (including weekly haematology and biochemistry samples) until Day 42 ('end of treatment' visit) or until resolution of any reversible toxicities; no follow-up visits are required thereafter. The results of the evaluations and observations, treatment for AEs/SAEs and a description of the reasons for stopping study treatment, should be recorded in the medical records and eCRF.

Patients who do not commence the lymphodepleting regimen and do not receive the 1RG-CART infusion will be followed at the Investigator's discretion according to routine clinical practice until resolution/stabilisation of any AEs related to trial-related procedures.

12 DEFINING THE END OF TRIAL

The 'End of Trial' is defined as the date when all patients have either withdrawn (see Section 11) from the trial or died, or the last patient to receive an infusion of 1RG-CART has completed the 24 month follow-up visit (following their last administration of 1RG-CART).

Patients remaining on trial at the time the End of Trial is declared will be re-enrolled and consented to a follow-on protocol which will continue to follow-up patients annually for at least 15 years after their last dose of 1RG-CART. This protocol will be independently submitted for MHRA and REC approval as required, at an appropriate time to ensure a seamless opening and transfer of patients following closure of the current trial.

It is the responsibility of the Sponsor to inform the MHRA and the Main REC within 90 days of the 'End of the Trial' that the trial has closed.

In cases of early termination of the trial (for example, due to toxicity) or a temporary halt by the Sponsor, the Sponsor will notify the MHRA and the Main REC within 15 days of the decision and a detailed, written explanation for the termination/halt will be given.

The entire trial will be stopped when:

- The drug is considered too toxic to continue treatment before the required number of patients have been recruited. Examples of unacceptable toxicity that could lead to premature termination of the trial include the following:
  - One fatal DLT in Dose Level 1;
  - Non-fatal DLTs in one or more patients in Dose Level 1;
  - Major toxicities (but not amounting to DLTs) in all or most patients in Dose Levels 1 to 5 without evidence of engraftment or anti-tumour efficacy.
- Failure of engraftment with no evidence of anti-tumour efficacy in Dose Levels 1 to 5 (i.e. expansion phase will not commence)
- The stated number of patients to be recruited is reached and the stated objectives of the trial are achieved or all patients have died.

Regardless of the reason for termination, all data available for the patient at the time of discontinuation of follow-up must be recorded in the eCRF. All reasons for discontinuation of treatment must be documented.

In terminating the trial, the Sponsor and the Investigator(s) must ensure that adequate consideration is given to the protection of the patient's interest.

13 DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

The final analysis will be conducted after one of the following conditions is met.

- The trial is terminated early (for example, due to toxicity).
- All patients have either withdrawn from the trial (see Section 11) or the last patient to receive an infusion of 1RG-CART has completed their 24 month follow-up visit (following their last administration of 1RG-CART).

Once one of the above conditions is met, a data cut-off date will be established. All patient visits occurring on or before this date will be analysed and summarised in the final CSR. Any data collected after this date will be summarised in supplemental reports.

13.1 Presentation of Data

Data will be presented in a descriptive fashion. Variables will be analysed to determine whether the criteria for the trial conduct are met. This will include a description of patients who did not meet all the eligibility criteria, an assessment of protocol violations, IMP accountability and other data that impact on the general conduct of the trial.

Baseline characteristics will be summarised for all enrolled patients. Patients who died or withdrew before treatment started or did not complete the required safety observations will be described and evaluated separately.

Treatment administration will be described for all patients who receive at least one dose of lymphodepleting treatment and/or 1RG-CART. Dose administration, dose modifications or delays and the duration of therapy will be described.

13.2 Feasibility of 1RG-CART Therapy

One of the primary objectives of the study is to assess the feasibility of 1RG-CART therapy in patients with relapsed or refractory neuroblastoma. This will be assessed as the percentage of patients entering the study (i.e. enrolled for leukapheresis / venepuncture) who undergo evaluation of 1RG-CART survival on or after Day 14. For 1RG-CART therapy to be considered feasible, it is proposed that at least 50% of patients must be assessed for 1RG-CART at this time point or later. This is a composite endpoint and failure to reach this assessment could be due to a number of factors, including but not limited to: failure of viral transduction, lack of viable transduced cells after the thawing, toxicity due to the conditioning regimen, early death or rapid disease progression.

For every patient who enters the trial (defined as enrolling for leukapheresis/venepuncture and modification of T-cells) and fails to undergo evaluation of 1RG-CART survival on or after Day 14, the reason for failure will be collected in the eCRF. The reasons will be collected as tick boxes (for the most likely reasons - disease progression, death, inadequate T-cell harvest etc) and free text.

13.3 Safety

Safety data will be collected from the date of first written consent. Safety variables will be summarised by descriptive statistics. Laboratory variables will be described using the NCI CTCAE Version 4.02.

Adverse events will be reported for each dose level and presented as tables of frequency of AEs by body system and by worse severity grade observed. Tables should indicate related and unrelated events.

13.4 Pharmacodynamics

13.4.1 Flow Cytometry Assay for Detection and Enumeration of 1RG-CART in Peripheral Blood

With this technique (see Section 8.1.1), the percentage of 1RG-CART present in each sample will be determined by flow cytometry gating on CD3+ cells that are additionally positive for QBEND10. Results will be expressed as % transduced T-cells present and/or absolute cell number.

All eligible patients who receive 1RG-CART and have at least one post-treatment blood sample taken on or after Day 14 will be included in the analysis.

13.4.2 [redacted]

[redacted]

13.4.3 [redacted]

[redacted]

13.4.4 [redacted]

[redacted]

13.4.5 [redacted]

[redacted]

[redacted]

13.4.6 [redacted]

[redacted]

13.4.7 [redacted]

[redacted]

13.4.8 [redacted]

[redacted]

13.5 Anti-Tumour Activity

Documenting anti-tumour activity is a secondary objective of this trial. Patients must have received 1RG-CART and completed a baseline and at least one post-treatment disease assessment to be evaluable for response. Patients who experience clear disease progression without a formal post-treatment disease assessment will be included and considered non-responders. Objective responses, the best tumour response achieved by each patient while on trial (according to each of the three response criteria) and the time to progression will be presented in the data listings.

13.6 Overall Survival and Progression-Free Survival

13.6.1 Overall Survival

All patients who receive 1RG-CART will be evaluable for overall survival, regardless of whether they subsequently receive other anti-cancer treatments. Overall survival will be measured from the date of administration of the first dose of 1RG-CART. Subgroup analyses of per protocol patients may be performed.

13.6.2 Progression-Free Survival

All patients who receive 1RG-CART and who complete a baseline and at least one post-treatment disease assessment (or experience clear disease progression without a formal post-treatment disease assessment) will be evaluable for progression-free survival, regardless of whether they subsequently receive other anti-cancer treatments. Progression-free survival will be measured from the date of administration of the first dose of 1RG-CART. This will be assessed according to all three tumour response criteria (RECIST 1.1, irRC and INRC). Since only limited follow-up (until Day 42) is planned for patients who receive the lymphodepleting regimen only, these patients will not be included in analyses of progression-free survival unless there is clear evidence of disease progression in the 42-day follow-up period or the patient's death is considered primarily due to the lymphodepleting regimen. Subgroup analyses of per protocol patients may be performed.

14 ADMINISTRATION

This trial is conducted under a clinical trial authorisation (CTA) and approval from the MHRA and the relevant REC(s) will be obtained before the start of this trial. This trial is sponsored and monitored by the Cancer Research UK, Centre for Drug Development. Applicable regulatory requirements are described in this section.

14.1 Protocol Deviations and Amendments

The protocol should be adhered to throughout the conduct of the trial, if a situation arises where the conduct of the trial may not be in line with the protocol, then site should contact the Sponsor to discuss this.

Amendments to the protocol may only be made with the approval of the Sponsor. A protocol amendment may be subject to review by the assigned Ethics Committee, Health Research Authority (HRA) and the MHRA. Written documentation of the Ethics Committee and HRA (and if appropriate the MHRA) 'favourable opinion' (i.e. approval) must be received before the amendment can be implemented and incorporated into the protocol if necessary.

14.2 Completion of the Electronic Case Report Form

Electronic case report forms (eCRFs) approved by the Sponsor will be used to collect the data. The Investigator is responsible for ensuring the accuracy, completeness, clarity and timeliness of the data reported in the eCRFs.

Only the Investigator and those personnel who have signed the Delegation Log provided by the Sponsor and have been authorised by the Investigator should enter or change data in the eCRFs. Authorised users will be included on a User Management Tool (UMT) in order to be provided access to the eCRF. All protocol required investigations, with the exception of the pharmacodynamic assays, must be reported in the eCRF. The Investigators must retain all original reports, traces and images from these investigations for future reference.

The collection and processing of personal data from the patients enrolled in this clinical trial will be limited to those data that are necessary to investigate the efficacy, safety, quality and usefulness of the study drug used in this trial. The data must be collected and processed with adequate precautions to ensure patient confidentiality and compliance with applicable data privacy protection according to the applicable regulations. The data collected will comply with Directive 95/46/EC of the European Parliament and of the Council of 24 October 1995 on the protection of individuals with regard to the processing of personal data.

Data will be entered directly into electronic screens by authorised site personnel. Amendments to eCRF data will be made directly to the system and the system audit trail will retain details of the original value(s), who made the change, a date and time, and a reason for the change.

Once an eCRF form has been entered by the site personnel, the data are cleaned using manual and automated checks. Queries will be issued electronically to the site. Authorised personnel must answer the queries by making relevant amendments to data or providing a response. Answered queries will be closed or reissued as appropriate.

Once the patient has completed or withdrawn from the trial and the eCRF has been fully completed, the Investigator must provide an electronic signature to authorise the complete subject casebook.

At the end of the trial all eCRFs are retained and archived by the Sponsor and a PDF copy provided to the Investigator who is responsible for archiving at site.

14.3 Trial Performance and Monitoring

Before the trial can be initiated, the prerequisites for conducting the trial must be clarified and the organisational preparations made with the trial centre. The Sponsor must be informed immediately of any change in the personnel involved in the conduct of the trial.

During the trial the Sponsor's CRA will be responsible for monitoring data quality in accordance with the Sponsor's SOPs. A strategic monitoring approach, including targeted source data verification, will be implemented where appropriate.

Before the trial start, the Investigator will be advised of the anticipated frequency of the monitoring visits. The Investigator will receive reasonable notification before each monitoring visit.

It is the responsibility of the CRA to:

- review trial records and compare them with source documents;
- check pharmacodynamic samples and storage;
- discuss the conduct of the trial and the emerging problems with the Investigator;
- check that the drug storage, dispensing and retrieval are reliable and appropriate; and
- verify that the available facilities remain acceptable.

It is the responsibility of the Sponsor to inform the MHRA and the main REC within 90 days of the 'End of the Trial' that the trial has closed (See definition in Section 12).

14.4 Source Document Verification

Unless agreed in writing, all data collected in the eCRF must be verifiable by the source data. Therefore, it is the Investigator's responsibility to ensure that both he/she and his/her trial team records all relevant data in the medical records. The Investigator must allow the CRA direct access to relevant source documentation for verification of data entered into the eCRF, taking into account data protection regulations. Entries in the eCRF will be compared with patients' medical records and the verification will be recorded in the eCRF.

Some source data may exist only electronically and be entered, or loaded directly into the eCRF. The patients' medical records, and other relevant data, may also be reviewed by appropriate qualified personnel independent from the Sponsor appointed to audit the trial, and by regulatory authorities. Details will remain confidential and patients' names will not be recorded outside the hospital.

14.5 Clinical Study Report

At appropriate intervals, interim data listings will be prepared to give the Investigator the possibility to review the data and check the completeness of information collected. All clinical data will be presented at the end of the trial on final data listings. The Sponsor will prepare a CSR based on the final data listings. The report will be submitted to the Investigator(s) for review and confirmation it accurately represents the data collected during the course of the trial. A summary of the final clinical report must be provided by the Sponsor to the MHRA and to the REC.

14.6 Record Retention

During the clinical trial and after trial closure the Investigator must maintain adequate and accurate records to enable both the conduct of a clinical trial and the quality of the data produced to be evaluated and verified. These essential documents (as detailed in Chapter V of Volume 10 [Clinical Trials] of The Rules Governing Medicinal Products in the European Union based upon Section 8 of the

ICH GCP Guidelines), including source documents such as scans, trial related documents and copies of the eCRFs, associated audit trail and SAE report forms, shall show whether the Investigator has complied with the principles and guidelines of Good Clinical Practice (GCP).

All essential documents required to be held by the Investigator must be stored in such a way that ensures that they are readily available, upon request, to the Regulatory Agency or Sponsor, for the minimum period required by national legislation or for longer if needed by the Sponsor. Records must not be destroyed without prior written approval from the Sponsor.

The medical files of trial subjects shall be retained in accordance with national legislation and in accordance with the maximum period of time permitted by the hospital, institution or private practice.

14.7 Ethical Considerations

Before starting the trial, the protocol, patient ICDs must go through the Sponsor's external review process, and be approved by the PSRB and receive the favourable opinion of the assigned REC.

It is the CI/PI's responsibility to update patients (or their authorised representatives, if applicable) whenever new information (in nature or severity) becomes available that might affect the patient's willingness to continue in the trial. The CI/PI must ensure this is documented in the patient's medical notes and the patient is re-consented.

The Sponsor and CI/PI must ensure that the trial is carried out in accordance with the GCP principles and requirements of the UK Clinical Trials regulations (SI 2004/1031 and SI 2006/1928 as amended), the ICH GCP guidelines and the Declaration of Helsinki (Appendix 5).

14.8 Indemnity

This trial is being carried out under the auspices of Cancer Research UK and therefore injury to a patient caused by the compounds under trial will not carry with it the right to seek compensation from the pharmaceutical industry. Cancer Research UK will provide patients with compensation for adverse side effects, in accordance with the principles set out in the Association of the British Pharmaceutical Industry (ABPI) guidelines on compensation for medicine-induced injury.

14.9 Publication Policy and Press Releases

Results of this trial must be submitted for publication. The Sponsor must be involved in reviewing all drafts of the manuscripts, abstracts, press releases and any other publications. Manuscripts must be submitted to the Sponsor at least 40 days in advance of being submitted for publication to allow time for the Sponsor to schedule a review and resolve any outstanding issues. Abstracts and press releases must also be submitted to the Sponsor at least 35 days in advance of being released.

Authors must acknowledge that the trial was sponsored by and performed with the support of the Sponsor.

The contribution of the Sponsor must be recognised by at least one member of staff being included as a co-author on each publication arising from the trial.

15 REFERENCES

Note for Guidance on Good Clinical Practice. ICH Topic E6. CSMP/ICH/135/95. EMEA, May 1996, updated September 1997 with post Step 4 errata included

Directive 2001/20/EC of the European Parliament and the Council of 4 April 2001 on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use. *Official Journal of the European Communities L121/34-44*

Detailed guidance for the request for authorisation of a clinical trial on a medicinal product for human use to the competent authorities, notification of substantial amendments and declaration of the end of the trial. **October 2005***. ENTR/F2/BL D(2003) CT 1 (Revision 2)*

Detailed guidance on the application format and documentation to be submitted in an application for an Ethics Committee opinion on the clinical trial on medicinal products for human use. **February 2006.** *ENTR/CT 2 (Revision 1)*

Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use. **April 2006.** *ENTR/CT 3 (Revision 2)*

The Medicines for Human Use (Clinical Trials) Regulations 2004 (Statutory Instrument 1031)

Commission Directive 2005/28/EC of 8 April 2005 laying down principles and detailed guidelines for good clinical practice as regards investigational medicinal products for human use, as well as the requirements for authorisation of the manufacturing or importation of such products. *Official Journal of the European Union L 91/13*

The Medicines for Human use (Clinical Trials) Amendment Regulations 2006 (Statutory Instrument 2006/1928).

Regulation (1394/2007/EC) of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation 726/2004/EC.

Detailed guidelines on good clinical practice specific to advanced therapy medicinal products 2009.

1. Skolnik JM, Barrett JS, Jayaramen B, Patel D, Adamson PC. 'Shortening the timeline of paediatric Phase I trials: The rolling six design.' J Clin Oncol 26 (2008), 190-195.

2. Davidoff AM, 'Neuroblastoma', *Semin Pediatr Surg,* 21 (2012), 2-14.

3. Colon NC, Chung DH. Neuroblastoma. *Advances in Pediatrics* (2011), 58: 207-311

4. Kaatsch P. Epidemiology of childhood cancer. *Cancer Treat Rev* (2010) Jun;36(4):277-85

5. Spix C, Pastore G, Sankila R, Stiller CA, Steliarova-Foucher E. 'Neuroblastoma incidence and survival in European children (1978-1997): report from the Automated Childhood Cancer Information System project'. *Eur J Cancer* (2006) Sep;42(13):2081-91.

6. Cohn SL et al., 'The International Neuroblastoma Risk Group (Inrg) Classification System: An Inrg Task Force Report', *J Clin Oncol,* 27 (2009), 289-97.

7. London WB et al., 'Clinical and Biologic Features Predictive of Survival after Relapse of Neuroblastoma: A Report from the International Neuroblastoma Risk Group Project', *J Clin Oncol,* 29 (2011), 3286-92.

8. Simon T et al., 'Treatment and outcomes of patients with relapsed, high-risk neuroblastoma: results of German trials', *Pediatr Blood Cancer* (2011) Apr;56(4):578-83.

9. Cheresh DA, Pierschbacher MD, Herzig MA, and Mujoo K, 'Disialogangliosides Gd2 and Gd3 Are Involved in the Attachment of Human Melanoma and Neuroblastoma Cells to Extracellular Matrix Proteins', *J Cell Biol,* 102 (1986), 688-96.

10. Mujoo K, Cheresh DA, Yang HM, and Reisfeld RA, 'Disialoganglioside Gd2 on Human Neuroblastoma Cells: Target Antigen for Monoclonal Antibody-Mediated Cytolysis and Suppression of Tumor Growth', *Cancer Res,* 47 (1987), 1098-104.

11. Schulz G et al., 'Detection of Ganglioside GD2 in Tumor Tissues and Sera of Neuroblastoma Patients', *Cancer Res,* 44 (1984), 5914-20.

12. Yuki N, Yamada M, Tagawa Y, Takahashi H, and Handa S, 'Pathogenesis of the Neurotoxicity Caused by Anti-Gd2 Antibody Therapy', *J Neurol Sci,* 149 (1997), 127-30.

13. Yamamoto et al., 'Heterogeneity in the Expression Pattern of Two Ganglioside Synthase Genes During Mouse Brain Development', *J Neurochem,* 66 (1996), 26-34.

14. Sondel PM, and Hank JA, 'Antibody-Directed, Effector Cell-Mediated Tumor Destruction', *Hematol Oncol Clin North Am,* 15 (2001), 703-21.

15. Kramer Ket al., 'Disialoganglioside G(D2) loss following monoclonal antibody therapy is rare in neuroblastoma', *Clin. Cancer Res*, 4 (1998), 2135–2139.

16. Kramer K et al., 'Disialoganglioside Gd2 Loss Following Monoclonal Antibody Therapy Is Rare in Neuroblastoma', *Med Pediatr Oncol,* 36 (2001), 194-6.

17. Schumacher-Kuckelkorn R, Hero B, Ernestus K, and Berthold F, 'Lacking Immunocytological GD2 Expression in Neuroblastoma: Report of 3 Cases', *Pediatr Blood Cancer,* 45 (2005), 195-201.

18. Parsons K, Bernhardt B, and Strickland B, 'Targeted Immunotherapy for High-Risk Neuroblastoma - the Role of Monoclonal Antibodies', *Ann Pharmacother,* 47 (2013), 210-8.

19. Yu AL et al., 'Anti-Gd2 Antibody with Gm-Csf, Interleukin-2, and Isotretinoin for Neuroblastoma', *N Engl J Med,* 363 (2010), 1324-34.

20. Unguru Y, Joffe S, Fernandez CV, and Yu AL, 'Ethical Issues for Control-Arm Patients after Revelation of Benefits of Experimental Therapy: A Framework Modeled in Neuroblastoma', *J Clin Oncol,* 31 (2013), 641-6.

21. Kalos M et al., 'T Cells with Chimeric Antigen Receptors Have Potent Antitumor Effects and Can Establish Memory in Patients with Advanced Leukemia', *Sci Transl Med,* 3 (2011), 95ra73.

22. Porter DL, Levine BL, Kalos M, Bagg A, and June CH, 'Chimeric Antigen Receptor-Modified T Cells in Chronic Lymphoid Leukemia', *N Engl J Med,* 365 (2011), 725-33.

23. Grupp SA et al., 'Chimeric Antigen Receptor-Modified T Cells for Acute Lymphoid Leukemia', *N Engl J Med,* 368 (2013), 1509-18.

24. Brentjens RJ et al., 'Safety and Persistence of Adoptively Transferred Autologous CD19-Targeted T Cells in Patients with Relapsed or Chemotherapy Refractory B-Cell Leukemias', *Blood,* 118 (2011), 4817-28.

25. Brentjens RJ et al., 'CD19-Targeted T Cells Rapidly Induce Molecular Remissions in Adults with Chemotherapy-Refractory Acute Lymphoblastic Leukemia', *Sci Transl Med,* 5 (2013), 177ra38.

26. Jensen MC et al., 'Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans', Biol. *Blood Marrow Transplant* (2010),16(9):1245–1256.

27. Pule MA et al., 'Virus-Specific T Cells Engineered to Coexpress Tumor-Specific Receptors: Persistence and Antitumor Activity in Individuals with Neuroblastoma', *Nat Med,* 14 (2008), 1264-70.

28. Louis CU et al., 'Antitumor Activity and Long-Term Fate of Chimeric Antigen Receptor-Positive T Cells in Patients with Neuroblastoma', *Blood,* 118 (2011), 6050-6.

29. Savoldo B et al., 'CD28 Costimulation Improves Expansion and Persistence of Chimeric Antigen Receptor-Modified T Cells in Lymphoma Patients', *J Clin Invest,* 121 (2011), 1822-6.

30. Straathof KC et al., 'Haemopoietic stem-cell transplantation with antibody-based minimal-intensity conditioning: a phase 1/2 study'. *Lancet* (2009),374(9693):912–920.

31. Strullu M et al., 'Allogeneic hematopoietic stem cell transplantation following reduced-intensity conditioning regimen in children: a single-center experience'. *Eur J Haematol* (2012) Jun;88(6):504-9.

32. Sung KW et al., 'Reduced-intensity allogeneic stem cell transplantation for children with neuroblastoma who failed tandem autologous stem cell transplantation'. *Pediatr Blood Cancer* (2011) Oct;57(4):660-5.

33. Cheung NK et al., 'Murine Anti-Gd2 Monoclonal Antibody 3f8 Combined with Granulocyte-Macrophage Colony-Stimulating Factor and 13-Cis-Retinoic Acid in High-Risk Patients with Stage 4 Neuroblastoma in First Remission', *J Clin Oncol,* 30 (2012), 3264-70.

34. Shusterman S et al., 'Antitumor activity of hu14.18-IL-2 in patients with relapsed/refractory neuroblastoma: a Children's Oncology Group (COG) phase II study.' *J. Clin. Oncol* (2010), 28, 4969–4975.

35. Maher J, 'Immunotherapy of Malignant Disease Using Chimeric Antigen Receptor Engrafted T Cells', *ISRN Oncol.*(2012).

36. Heslop HE, 'Safer Cars', *Mol Ther,* 18 (2010), 661-2.

37. Morgan RA et al., 'Case Report of a Serious Adverse Event Following the Administration of T Cells Transduced with a Chimeric Antigen Receptor Recognizing Erbb2', *Mol Ther,* 18 (2010), 843-51.

38. Davila ML et al., 'Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia.' *Sci Transl Med* (2014); 6, 224ra25: 1-10.

39. Brentjens R, Yeh R, Bernal Y, Riviere I, and Sadelain M, 'Treatment of Chronic Lymphocytic Leukemia with Genetically Targeted Autologous T Cells: Case Report of an Unforeseen Adverse Event in a Phase I Clinical Trial', *Mol Ther,* 18 (2010), 666-8.

40. Warren EH et al., 'Therapy of Relapsed Leukemia after Allogeneic Hematopoietic Cell Transplantation with T Cells Specific for Minor Histocompatibility Antigens', *Blood,* 115 (2010), 3869-78.

41. Johnson LA et al., 'Gene Therapy with Human and Mouse T-Cell Receptors Mediates Cancer Regression and Targets Normal Tissues Expressing Cognate Antigen', *Blood,* 114 (2009), 535-46.

42. Cameron BJ et al., 'Identification of a Titin-Derived Hla-A1-Presented Peptide as a Cross-Reactive Target for Engineered Mage A3-Directed T Cells', *Sci Transl Med,* 5 (2013), 197ra03.

43. Lamers CH et al., 'Treatment of Metastatic Renal Cell Carcinoma with Caix Car-Engineered T Cells: Clinical Evaluation and Management of on-Target Toxicity', *Mol Ther,* 21 (2013), 904-12.

44. Parkhurst, MR, et al., 'T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis', *Mol Ther*, 2011. 19(3): p. 620-6.

45. Lamers CH et al., 'Immune Responses to Transgene and Retroviral Vector in Patients Treated with Ex Vivo-Engineered T Cells', *Blood,* 117 (2011), 72-82.

46. Maus VM et al., 'T cells expressing chimeric antigen receptors can cause anaphylaxis in humans'. *Cancer Immunol Res* (2013), 1, 26-31.

47. Beatty GL et al., 'Mesothelin-Specific Chimeric Antigen Receptor mRNA-Engineered T cells Induce Antitumor Activity in Solid Malignancies', *Cancer Immunol Res* (2014), 2 (2), 112-20.

48. Rosado FG, and Kim AS, 'Hemophagocytic Lymphohistiocytosis: An Update on Diagnosis and Pathogenesis', *Am J Clin Pathol,* 139 (2013), 713-27.

49. Straathof KCM et al., 'Treatment of nasopharyngeal carcinoma with Epstein-Barr virus-specific T lymphocytes'. *Blood,* (2005),105 (5), 1898–1904.

50. Bollard CM et al., 'Cytotoxic T lymphocyte therapy for Epstein-Barr virus+ Hodgkin's disease', *J. Exp. Med.* (2004), 200 (12), 1623–1633.

51. Kochenderfer JN et al., 'Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologus T cells genetically engineered to recognize CD19', *Blood*, 116 (2010), 581-7.

52. Kochenderfer JN et al., 'B-cell depletion and remissions of malignancy along with cytokine-associated toxicity in a clinical trial of anti-CD19 chimeric-antigen-receptor-transduced T cells', *Blood*, 119 (2012), p2709-20.

53. Eisenhauer EA et al., 'New response evaluation criteria in solid tumours: Revised RECIST guideline (version 1.1.)', *European Journal of Cancer* (2009), 45:2, 228-247.

54. Wolchok JD et al., 'Guidelines for the Evaluation of Immune Therapy Activity in Solid Tumours: Immune-Related Response Criteria', *Clin Cancer Res* (2009), 15, 7412-7420.

55. Brodeur GM et al., 'Revisions of the International Criteria for Neuroblastoma Diagnosis, Staging and Response to Treatment', *J Clin Oncol*, 11 (1993), 1466-1477.

56. Lee DW et al., 'Current concepts in the diagnosis and management of cytokine release syndrome', *Blood* 10 July 2014 Vol 124 (2): 188-195.

57. Wang Y, et al., 'Effective response and delayed toxicities of refractory advanced diffuse large B-cell lymphoma treated by CD20-directed chimeric antigen receptor-modified T cells', *Clin Immunol*, 2014. **155**(2): p. 160-75.

58. Papadopoulou A, et al., 'Systemic inflammatory response syndrome after administration of unmodified T lymphocytes', *Mol Ther*, 2014. **22**(6): p. 1134-8.

59. Wu ZL, et al., 'Expression of GD2 ganglioside by untreated primary human neuroblastomas', *Cancer Res*, 1986. **46**(1): p. 440-3.

60. Schengrund CL and Shochat SJ, 'Gangliosides in neuroblastomas', *Neurochem Pathol*, 1988. **8**(3): p. 189-202.

61. Kochenderfer JN et al., 'Donor-derived CD19-targeted T cells cause regression of malignancy persisting after allogeneic hematopoietic stem cell transplantation.' *Blood*, 2013.

62. Kochenderfer JN et al., 'Chemotherapy-Refractory Diffuse Large B-Cell Lymphoma and Indolent B-Cell Malignancies Can Be Effectively Treated With Autologous T Cells Expressing an Anti-CD19 Chimeric Antigen Receptor.' *J Clin Oncol*, 2014.

63. Heslop HE et al., 'Long-term outcome of EBV-specific T-cell infusions to prevent or treat EBV-related lymphoproliferative disease in transplant recipients.' *Blood*, 2010. **115**(5): p. 925-35

64. Park JH, et al., 'Phase I trial of autologous CD19-targeted CAR-modified t cells as consolidation after purine analog-based first-line therapy in patients with previously untreated CLL.' *J Clin Oncol* 2014. **32**(5s): p. abstr 7020.

65. Ramos CA et al., 'Effect of a costimulatory endodomain on the performance of T cells expressing CD19-directed chimeric antigen receptors (CARs) in subjects with relapsed/refractory B-cell malignancies.' *J Clin Oncol* 2011. **29**(abstract 2541).

66. Slovin S et al., 'Chimeric antigen receptor (CAR+) modified T cells targeting prostate-specific membrane antigen (PSMA) in patients (pts) with castrate metastatic prostate cancer (CMPC).' *J Clin Oncol* 2013. **suppl6**(abstract 72).

67. Ritchie, D.S., et al., 'Persistence and efficacy of second generation CAR T cell against the LeY antigen in acute myeloid leukemia.' *Mol Ther*, 2013. **21**(11): p. 2122-9.

68. Morgan RA et al., 'Cancer regression and neurological toxicity following anti-MAGE-A3 TCR gene therapy.' *J Immunother*, 2013. **36**(2): p. 133-51.

69. Maude S et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med.* 2014. **371**: 1507-1517.

16 APPENDICES

16.1 APPENDIX 1: CYTOKINE RELEASE SYNDROME REVISED GRADING SYSTEM

(From Lee et al., 2014 [56])

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

[1] Grades 2-4 refer to NCI CTCAE Version 4.02 grading.
[2] High dose vasopressors include:

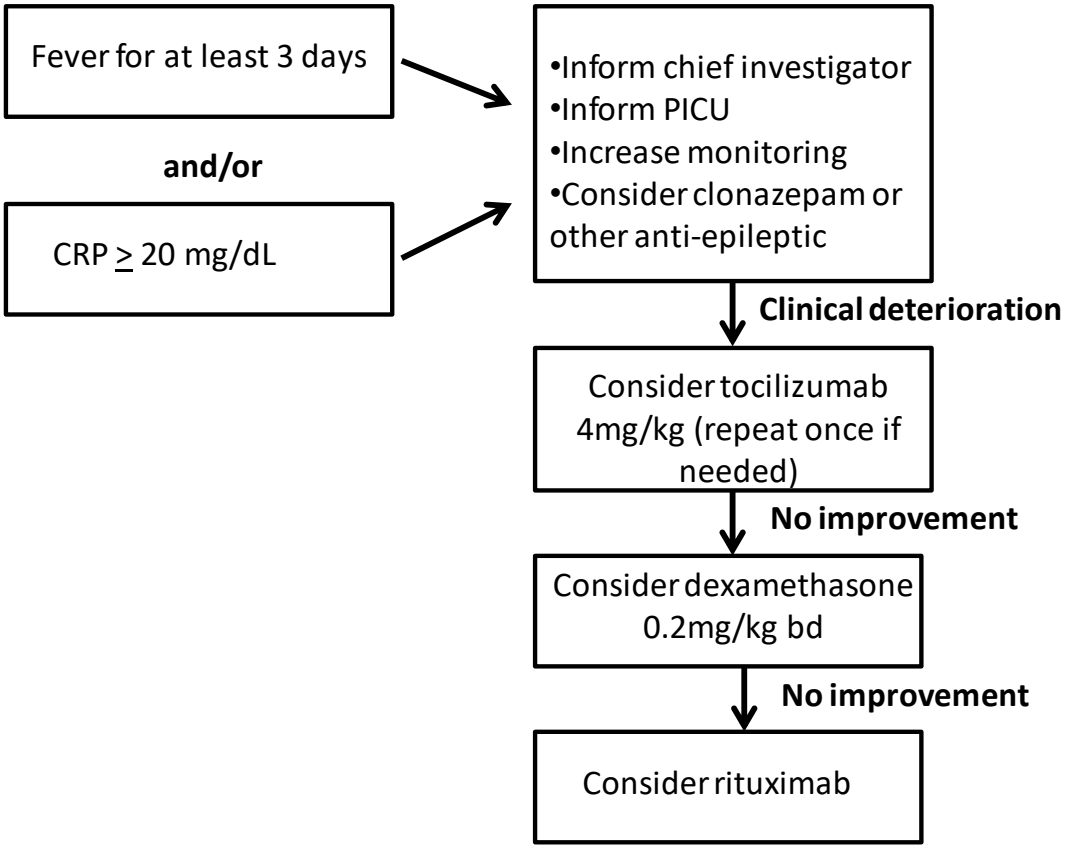
- norepinephrine monotherapy ≥20 μg/kg/min,
- dopamine monotherapy ≥10 μg/kg/min,
- phenylephrine monotherapy ≥200 μg/kg/min,
- epinephrine monotherapy ≥10 μg/kg/min.

16.2 APPENDIX 2: INDICATORS AND MANAGEMENT OF SEVERE CYTOKINE RELEASE SYNDROME (DERIVED FROM DAVILA ET AL, 2014 [38])

Indicators of severe cytokine release secondary to CD19-directed CAR T-cells in ALL

- Fever for at least three consecutive days.
- 2 cytokines increase at least 75-fold over baseline or one cytokine increases at least 250-fold.
- At least one clinical sign of toxicity such as
    - Hypotension requiring at least one intravenous vasopressor;
    - Hypoxia (pO2 <90%);
    - Neurological disorders (such as mental status changes, obtundation or seizures).

Suggested management algorithm for patients receiving 1RG-CART



16.3 APPENDIX 3: LANSKY PLAY PERFORMANCE SCALE

This performance scale is recommended for children aged <16 years:

Fully active and normal	100
Minor restrictions in physically strenuous activity	90
Active, but tires more easily	80
Both greater restriction of, and less time spent in, active play	70
Up and around, but minimal active play; keeps busy with quieter activities	60
Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities	50
Mostly in bed; participates in quiet activities	40
In bed; needs assistance even for quiet play	30
Often sleeping; play entirely limited to very passive activities	20
No play; does not get out of bed	10
Unresponsive	0

16.4 APPENDIX 4: KARNOFSKY PERFORMANCE SCALE

This performance scale is recommended for children aged ≥16 years:

Normal, no complaints, no signs of disease	100
Capable of normal activity, few symptoms or signs of disease	90
Normal activity with some difficulty, some symptoms or signs	80
Caring for self, not capable of normal activity or work	70
Requiring some help, can take care of most personal requirements	60
Requires help often, requires frequent medical care	50
Disabled, requires special care and help	40
Severely disabled, hospital admission indicated but no risk of death	30
Very ill, urgently requiring admission, requires supportive measures or treatment	20
Moribund, rapidly progressing fatal disease processes	10
Death	0

16.5 APPENDIX 5: DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964, and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington 2002 (Note of Clarification on paragraph 29 added)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30 added)
59th WMA General Assembly, Seoul, October 2008

A. INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects**,** including research on identifiable human material and data.

   The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.

2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human subjects to adopt these principles.

3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

4. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

5. Medical progress is based on research that ultimately must include studies involving human subjects. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.

6. In medical research involving human subjects, the well-being of the individual research subject must take precedence over all other interests.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.

8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is subject to ethical standards that promote respect for all human subjects and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.

10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

B. PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects.

12. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human subjects must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for subjects and provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study subjects to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human subjects must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional. The responsibility for the protection of research subjects must always rest with the physician or other health care professional and never the research subjects, even though they have given consent.
17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first subject.
20. Physicians may not participate in a research study involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human subjects may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research subjects.
22. Participation by competent individuals as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human subjects, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential

subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information. After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.

26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.

27. For a potential research subject who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential subject, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.

28. When a potential research subject who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential subject's dissent should be respected.

29. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the subject or a legally authorized representative.

30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

32. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

    - The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
    - Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the

patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

34. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
35. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
36. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

16.6 APPENDIX 6: ASSESSMENT OF RESPONSE USING RECIST 1.1 CRITERIA

The following guidelines are based on the publication by E.A. Eisenhauer et al. (European Journal of Cancer 2009, **45:** 228-247) [53]. Please refer to the full publication for further details.

1. Measurability of Tumour at Baseline

1.1. Definitions

At baseline, tumour lesions/lymph nodes will be categorised measurable or non-measurable as follows:

1.1.1. Measurable

Tumour lesions: Must be accurately measured in at least one dimension (longest diameter in the plane of measurement is to be recorded) with a minimum size of:

- 10 mm by CT scan or MRI (CT scan slice thickness no greater than 5 mm).
- 10 mm calliper measurement by clinical exam (lesions which cannot be accurately measured with callipers should be recorded as non-measurable).
- 20 mm by chest X-ray

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be 15mm in the short axis when assessed by CT scan or MRI (CT scan slice thickness recommended to be no greater than 5 mm). Nodes that have a short axis <10 mm are considered non-pathological and should not be recorded or followed. At baseline and in follow-up, only the short axis will be measured and followed.

1.1.2. Non-Measurable

All other lesions, including small lesions (longest diameter <10 mm or pathological lymph nodes with 10 to <15 mm short axis) as well as truly non-measurable lesions. Lesions considered truly non-measurable include: leptomeningeal disease, ascites, pleural or pericardial effusion, inflammatory breast disease, lymphangitic involvement of skin or lung, abdominal masses/abdominal organomegaly identified by physical exam that is not measurable by reproducible imaging techniques.

1.1.3. Special Considerations Regarding Lesion Measurability

Bone lesions:

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

Cystic lesions:

- Lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

- ‘Cystic lesions’ thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Lesions with prior local treatment:

- Tumour lesions situated in a previously irradiated area, or in an area subjected to other loco-regional therapy, are usually not considered measurable unless there has been demonstrated progression in the lesion. Study protocols should detail the conditions under which such lesions would be considered measurable.

1.2. Specifications by Methods of Measurements

1.2.1. Measurement of Lesions

All measurements should be recorded in metric notation, using callipers if clinically assessed. All baseline evaluations should be performed as close as possible to the treatment start.

1.2.2. Method of Assessment

The same method of assessment and the same technique should be used to characterise each identified and reported lesion at baseline and during follow-up, wherever possible. Imaging based evaluation should always be done rather than clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Ultrasound:

Ultrasound is not considered useful in assessment of lesion size and should not be used as a method of measurement. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised.

Tumour markers (urinary catecholamines):

Tumour markers (i.e. urinary catecholamines) alone cannot be used to assess objective tumour response. If markers are initially above the upper normal limit, however, they must normalise for a patient to be considered in complete response.

Cytology, histology:

Bone marrow examination can be used to differentiate between a PR and CR in patients with no residual macroscopic disease elsewhere.

2. Tumour Response Evaluation

2.1 Assessment of Overall Tumour Burden and Measurable Disease

To assess objective response or progression, it is necessary to estimate the overall tumour burden at baseline and use this as a comparator for subsequent measurements. Measurable disease is defined by the presence of at least one measurable lesion (as detailed above in Section 1).

2.2. Baseline Documentation of ‘Target’ and ‘Non-Target’ Lesions

When more than one measurable lesion is present at baseline all lesions up to a maximum of five lesions total (and a maximum of two lesions per organ) representative of all involved organs should be identified as target lesions (this means in instances where patients have only one or two organ sites involved a maximum of two and four lesions respectively will be included in RECIST response assessment). Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements.

For RECIST 1.1 response assessment, the sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum

diameters. If lymph nodes are to be included in the sum, then, only the short axis is added into the sum. All other lesions (or sites of disease) including pathological lymph nodes should be identified as non-target lesions. These lesions should be followed as 'present', 'absent', or as 'unequivocal progression'. Multiple non-target lesions involving the same organ may be considered a single item for RECIST response assessment (e.g. 'multiple enlarged pelvic lymph nodes' or 'multiple liver metastases').

2.3. Response Criteria

2.3.1. Evaluation of Target Lesions

- Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

- Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.

- Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progression).

- Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

2.3.2. Special Notes on the Assessment of Target Lesions

Lymph nodes.

Lymph nodes identified as target lesions should always have the actual short axis measurement recorded (measured in the same anatomical plane as the baseline examination), even if the nodes regress to below 10 mm on study. This means that when lymph nodes are included as target lesions, the 'sum' of lesions may not be zero even if complete response criteria are met, since a normal lymph node is defined as having a short axis of <10 mm.

Target lesions that become 'too small to measure'.

While on study, all lesions (nodal and non-nodal) recorded at baseline should have their actual measurements recorded at each subsequent evaluation, even when very small (e.g. 2 mm). However, sometimes lesions or lymph nodes which are recorded as target lesions at baseline become so faint on imaging that the radiologist may not feel comfortable assigning an exact measure and may report them as being 'too small to measure'. When this occurs it is important that a value be recorded on the case report form. If it is the opinion of the radiologist that the lesion has likely disappeared, the measurement should be recorded as 0 mm. If the lesion is believed to be present and is faintly seen but too small to measure, a default value of 5 mm should be assigned.

Lesions that split or coalesce on treatment:

When non-nodal lesions 'fragment', the longest diameters of the fragmented portions should be added together to calculate the target lesion sum. Similarly, as lesions coalesce, a plane between them may be maintained that would aid in obtaining maximal diameter measurements of each individual lesion. If the lesions have truly coalesced such that they are no longer separable, the vector of the longest diameter in this instance should be the maximal longest diameter for the 'coalesced lesion'.

2.3.3. Evaluation of Non-Target Lesions

- Complete Response (CR): Disappearance of all non-target lesions and normalisation of tumour marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

- Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumour marker level above the normal limits.
- Progressive Disease (PD): Unequivocal progression of existing non-target lesions. (Note: the appearance of one or more new lesions is also considered progression; also a modest 'increase' in the size of one or more non-target lesions is usually not sufficient to qualify for unequivocal progression status in patients who also have measurable disease).

2.3.4. New Lesions

The unequivocal appearance of new malignant lesions (i.e. not attributable to differences in scanning technique, change in imaging modality etc) denotes disease progression. A lesion identified on a follow-up study in an anatomical location that was not scanned at baseline is considered a new lesion and indicates disease progression. If a new lesion is equivocal, for example because of its small size, continued therapy and follow-up evaluation will clarify if it represents truly new disease. If repeat scans confirm there is definitely a new lesion, then progression should be declared using the date of the initial scan.

2.4. Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the study treatment until the end of treatment taking into account any requirement for confirmation.

Table 1 and Table 2 provide a summary of the overall response status calculation at each time point for patients who have measurable disease at baseline (Table 1) or non-measurable (therefore non-target) disease only (Table 2). Note that when no imaging/measurement is done at all at a particular time point, the patient is classified not evaluable (NE) at that time point. If only a subset of lesion measurements are made at an assessment, usually this is also considered NE at that time point, unless a convincing argument can be made that the contribution of the individual missing lesion(s) would not change the assigned time point response (for example, when there is clear evidence of disease progression). Table 3 shows how the best overall response is the derived from the response at each time point. For this trial, to achieve a best response of SD measurements must have met the SD criteria at least once after study entry at least 6 weeks after infusion of 1RG-CART. Also, for this trial, to achieve a CR according to RECIST 1.1 criteria, the patient must have at least one documented disease-free bone marrow post-baseline unless the bone marrow was clear at baseline.

Table 1 – Time Point Response: Patients with Target (+/–Non-Target) Disease

Target lesions	Non-target lesions	New lesions	Overall response
CR	CR	No	CR
CR	Non-CR/non-PD	No	PR
CR	Not evaluated	No	PR
PR	Non-PD or not all evaluated	No	PR
SD	Non-PD or not all evaluated	No	SD
Not all evaluated	Non-PD	No	NE
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = not evaluable.

Table 2 – Time Point Response: Patients with Non-Target Disease Only

Non-target lesions	New lesions	Overall response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD(a)

Not all evaluated	No	PD
Unequivocal PD	Yes or No	PD
Any	Yes	PD

CR = complete response, PD = progressive disease.
(a) 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised.

Best response determination in trials where confirmation of complete or partial response is required: Complete or partial responses may be claimed only if the criteria for each are met at a subsequent time point as specified in the protocol (generally 4 weeks later). In this circumstance, the best overall response can be interpreted as in Table 3

Table 3 – Best Overall Response

Overall response	Overall response	BEST overall response
First time point	Subsequent time point(s)	
CR	CR	CR
CR	PR	SD, PD or PR(a)
CR	SD	SD provided minimum criteria for SD duration met, otherwise, PD
CR	PD	SD provided minimum criteria for SD duration met, otherwise PD
CR	NE	SD provided minimum criteria for SD duration met, otherwise NE
PR	CR	PR
PR	PR	PR
PR	SD	SD
PR	PD	SD provided minimum criteria for SD duration met, otherwise, PD
PR	NE	SD provided minimum criteria for SD duration met, otherwise NE
NE	NE	NE

CR = complete response, PR = partial response, SD = stable disease, PD = progressive disease, and NE = inevaluable.
(a) If a CR is truly met at first time point, then any disease seen at a subsequent time point, even disease meeting PR criteria relative to baseline, makes the disease PD at that point (since disease must have reappeared after CR). Best response would depend on whether minimum duration for SD was met. However, sometimes 'CR' may be claimed when subsequent scans suggest small lesions were likely still present and in fact the patient had PR, not CR at the first time point. Under these circumstances, the original CR should be changed to PR and the best response is PR. Note that for this trial, to achieve an overall best response of CR, a patient must have a documented disease-free bone marrow post-baseline unless the bone marrow was clear at screening.

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as 'symptomatic deterioration'.

2.4.1. Duration of Overall Response

The duration of overall response is measured from the time measurement criteria are first met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded on study).

The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

2.4.2. Duration of Stable Disease

Stable disease is measured from the start of the treatment (i.e. infusion of 1RG-CART) until the criteria for progression are met, taking as reference the smallest sum on study (if the baseline sum is the smallest, this is the reference for calculation of PD).

16.7 APPENDIX 7: ASSESSMENT OF RESPONSE USING IMMUNE-RELATED RESPONSE CRITERIA (irRC)

The following guidelines are based on the publication by JD Wolchok et al. (Clin Cancer Res. 2009 Dec 1;15(23):7412-20) [54]. Please refer to the full publication for further details.

Assessment of tumour burden

Immune-related response criteria are based on WHO criteria in that bidimensional measurements are required (unlike RECIST criteria, which involve unidimensional measurements only). For irRC, only index and measurable new lesions are taken into account (in contrast to RECIST criteria, which do not require the measurement of new lesions, nor do they include new lesion measurements in the characterisation of evolving tumour burden). At the baseline tumour assessment, the sum of the products of the two largest perpendicular diameters (SPD) of all index lesions (five lesions per organ, up to 10 visceral lesions and five cutaneous index lesions) is calculated. At each subsequent tumour assessment, the SPD of the index lesions and of new, measurable lesions (≥5 x 5 mm; up to 5 new lesions per organ; 5 new cutaneous lesions and 10 visceral lesions) are added together to provide the total tumour burden:

Tumour burden = $SPD_{index\ lesions}$ + $SPD_{new,\ measurable\ lesions}$

In irRC, new measurable lesions (i.e. ≥5 x 5mm) are incorporated into tumour burden, whereas according to RECIST criteria new measurable lesions (i.e. longest diameter ≥ 10 mm) always represent PD. In irRC, new non-measurable lesions (i.e. ≤5 x 5mm) do not define progression (but preclude irCR), whereas according to RECIST criteria new non-measurable lesions (i.e. longest diameter <10 mm) always represent PD. Although not specified in the irRC described by Wolchok et al., for this trial it is recommended that to be considered an index lesion (i.e. a measurable lesion at baseline), a lesion must measure at least 5 x 5mm.

Time-point response assessment using irRC

Percentage changes in tumour burden per assessment time point describe the size and growth kinetics of both conventional and new, measurable lesions as they appear. At each tumour assessment, the response in target and new, measurable lesions is defined based on the change in tumour burden (after ruling out irPD). Decreases in tumour burden must be assessed relative to baseline measurements (i.e. the SPD of all index lesions at screening).

Whereas RECIST criteria has separate definitions of tumour response for target lesion and non-target lesions, irRC defines tumour response for tumour burden (index and new measurable lesions together). The irRC tumour response definitions for any time point are as follows:

Time Point Response	IrRC definition
CR	Disappearance of all lesions and no new lesions
PR	≥50% decrease in tumour burden compared with baseline
SD	50% decrease in tumour burden compared with baseline cannot be established nor 25% increase compared with nadir
PD	At least 25% increase in tumour burden compared with nadir

Overall response using the irRC

The overall response according to the irRC is derived from time-point response assessments (based on tumour burden) as follows:

- irCR, complete disappearance of all lesions (whether measurable or not, and no new lesions); confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented.
- irPR, decrease in tumour burden ≥50% relative to baseline confirmed by a consecutive assessment at least 4 weeks after first documentation.
- irSD, not meeting criteria for irCR or irPR, in absence of irPD.
- irPD, increase in tumour burden ≥25% relative to nadir (minimum recorded tumour burden); confirmation by a repeat, consecutive assessment no less than 4 weeks from the date first documented.

Derivation of irRC overall responses:

Measurable response	**Non-measurable response**		**Overall response**
Index and new, measurable lesions (tumour burden), *%	**Non-index lesions**	**New, non-measurable lesions**	
↓100	Absent	Absent	irCR†
↓100	Stable	Any	irPR†
↓100	Unequivocal progression	Any	irPR†
↓≥50	Absent / stable	Any	irPR†
↓≥50	Unequivocal progression	Any	irPR†
↓<50 to <25↑	Absent / stable	Any	irSD
↓<50 to <25↑	Unequivocal progression	Any	irSD
≥25?	Any	Any	irPD†

*Decreases assessed relative to baseline, including measurable lesions only (>5 × 5 mm).
†Assuming response (irCR or irPR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 wk apart. Note that for this trial, to achieve an irRC overall response of CR, a patient must have a documented disease-free bone marrow post-baseline unless the bone marrow was clear at baseline

Note that, according to irRC, patients are considered to have irPR or irSD even if new lesions are present, as long as they meet the respective thresholds of response as described above. Furthermore, patients are not considered to have irPD if new lesions are present but the tumour burden of all lesions does not increase by ≥25%. In contrast to irCR, irPR, and irPD, a response of irSD does not require confirmation. It is important to note that irCR, irPR, and irSD include all patients with CR, PR, or SD by WHO criteria (many of whom would also have CR, PR or SD by RECIST criteria) as well as some patients with WHO-determined or RECIST-determined PD. Patients with irSD according to irRC, particularly those with slow-declining tumour burden ≥25% from baseline at the last tumour assessment, may be considered clinically meaningful because they show an objectively measurable reduction in tumour burden without reaching the 50% threshold that defines irPR.

Confirmation of progression

Note that unlike RECIST and WHO criteria, if a patient is classified as having irPD at a post-baseline tumour assessment, then confirmation of irPD by a second scan in the absence of rapid clinical deterioration is required. Confirmation of progression requires an increase in tumour burden ≥25%

compared with the nadir at two consecutive time points at least 4 weeks apart. It is recommended that this be done at the discretion of the investigator because follow-up with observation alone may not be appropriate for patients with a rapid decline in performance status.

16.8 APPENDIX 8: ASSESSMENT OF RESPONSE USING INTERNATIONAL NEUROBLASTOMA RESPONSE CRITERIA (INRC)

The following guidelines are based on the publication by GM Brodeur et al. (J Clin Oncol 1993, 11:1466-1477) [55]. Please refer to the full publication for further details.

The response definitions of the INRC are summarised in the bullet points and Table below. Note that the INRC recommends that the **volume** of the primary tumour and large metastases be determined to the best approximation (i.e. using 3-dimensional measurements), and that the volume should be used to determine response. This differs significantly from irRC criteria (in which the sum of products of bidimensional lesions is used) and RECIST criteria (in which the sum of unidimensional measurements is used). In this trial, in order to simplify data entry and verification, and to facilitate data analysis, only bidimensional lesion measurements will be used in assessing response according to INRC. No recommendations are made in the INRC criteria regarding minimum size, number of lesions to measure, lesion location etc. Therefore, in this trial, in order to simplify data entry and verification, and to facilitate data analysis, the same lesions and lesion measurements will be for assessing INRC response as used for assessing irRC response.

- A complete response (CR) indicates complete disappearance of all primary and metastatic disease, and the normalisation of catecholamines and metabolites (if they were increased at diagnosis).

- A very good partial response (VGPR) indicates a 90% to 99% volume reduction in the primary tumour, with clearing of all measurable metastatic disease and normalisation of catecholamines. The only exception to this would be residual abnormalities on technetium bone scan attributable to incomplete healing of the bone. The MIBG scan (if performed) should be negative at all metastatic sites.

- A partial response (PR) indicates a greater than 50% volume reduction of the primary tumour and a greater than 50% reduction of all measurable metastatic sites (but not satisfying criteria for CR or VGPR). Residual positive marrow aspirate or biopsy (one site) is permitted if this reflects a decrease from the number of sites of involvement at the time of diagnosis (e.g., two to four sites positive initially). MIBG scans may allow residual tumour to be distinguished, but if this is not available, there should be no new bone lesions, and the pre-existing lesions should be improving. Note that this definition is somewhat more stringent than World Health Organisation (WHO), RECIST and irRC definitions of PR.

- The term mixed response (MR) is used to indicate a greater than 50% response at one or more sites and a less than 50% reduction at one or more other sites. This might occur, for instance, if metastatic disease responded, but the primary tumour size did not change. In such a case, the histology of the tumour could have changed from neuroblastoma either to ganglioneuroma or to predominantly scar tissue and necrosis. In this situation, a surgical procedure could convert such a patient status to PR, VGPR, or even CR. Note that at least some cases of mixed response (MR) according to INRC would likely to be categorised as partial response (PR) according to other response criteria.

- No response (NR) indicates a less than 50% reduction of some or all measurable lesions, but no increase of greater than 25% in these lesions and no new lesions. This definition corresponds to the definition of SD according to WHO and irRC criteria.

- Progressive disease (PD) indicates a greater than 25% increase in any pre-existing lesion or any new lesion.

According to INRC, determination of overall response is based on the response of the primary tumour and all metastatic sites. Thus, complete disappearance of all metastatic disease, but only a PR at the primary site would represent a PR overall. However, if all residual tumour tissue had become mature ganglioneuroma or was resectable, the chemotherapy response would be a PR, but the response to combined therapy would be a CR. For this trial, a response to 1RG-CART that results in residual

mature ganglioneuroma or residual disease that is subsequently surgically resected, will be considered a PR or VGPR and not a CR.

Response	**Primary Tumour**	**Metastatic Sites***
Complete Response (CR)	No Tumour	- No tumour - Catecholamines normal
Very Good Partial Response (VGPR)	Decrease by 90 - 99%	- No tumour - Residual bone changes allowed
Partial Response (PR)	Decreased by >50%	- All measurable sites decreased by > 50% - Bones and bone marrow: number of positive sites decreased by > 50%; no more than one positive bone marrow site allowed*
Mixed Response (MR)	- No new lesions - >50% reduction of any measurable lesion (primary or metastases) with ≤50% reduction in any other - <25% increase in any existing lesion	
No response (NR)	- No new lesions - ≤50% reduction but <25% increase in any existing lesion	
Progressive Disease (PD)	- Any new lesion - increase of any measurable lesion by ≥25% - Previous negative marrow biopsy for tumour.	

*One positive marrow aspirate or biopsy allowed for PR if this represents a decrease from the number of positive sites at diagnosis.

Duration of response

According to INRC, tumours that respond initially (e.g. after 2 months) but grow back by 4 months would be considered to have an overall response of PD. However, for this trial, in which a novel experimental therapy is being evaluated, transient responses are still of clinical interest. Therefore, to be considered an INRC response (CR, VGPR, PR) in this trial, response only has to be sustained for at least 4 weeks.

16.9 APPENDIX 9: ASSESSMENT AND MANAGEMENT OF PAIN

Neuropathic-type pain has been regularly reported in children receiving GD2-targeted antibodies, so it is possible that similar pain could be experienced following 1RG-CART infusion or during the T-cell expansion phase in the weeks following 1RG-CART infusion. The following measures should be in place to detect and manage pain:

- A daily age-appropriate pain score will be recorded from Day 0 to Day 14. Thereafter a pain score (Wong-Baker FACES® Pain Rating Scale) will be recorded as clinically indicated.
- In the event of worsening pain, if it appears likely to be unrelated to infused 1RG-CART, escalate through standard pain management as per local Haemato/Oncology supportive care guidelines.
- If the pain is suspected to be related to the infused 1RG-CART:

    1. Start gabapentin 10 mg/kg (Day 1: 10 mg/kg once daily, Day 2: 10 mg/kg twice daily, Day 3: 10 mg/kg three times daily, per oral/nasogastric tube) and continue
    2. Alert the Pain Team
    3. For mild pain, treat initially with paracetamol or codeine phosphate
    4. For severe pain consider a morphine infusion and titrate rate against response.

16.10 APPENDIX 10: ESTIMATION OF BODY SURFACE AREA IN INFANTS AND CHILDREN

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Estimation of Body-Surface Area in Infants and Children*

Body Weight (kg)	Surface Area (m²)
2	0.16
2.5	0.19
3	0.21
3.5	0.24
4	0.26
4.5	0.28
5	0.3
5.5	0.32
6	0.34
6.5	0.36
7	0.38
7.5	0.4
8	0.42
8.5	0.44
9	0.46
9.5	0.47
10	0.49

Body Weight (kg)	Surface Area (m²)
11	0.53
12	0.56
13	0.59
14	0.62
15	0.65
16	0.68
17	0.71
18	0.74
19	0.77
20	0.79
21	0.82
22	0.85
23	0.87
24	0.9
25	0.92
26	0.95
27	0.97
28	1.0
29	1.0
30	1.1
31	1.1
32	1.1
33	1.1
34	1.1
35	1.2
36	1.2
37	1.2
38	1.2
39	1.3
40	1.3

Body Weight (kg)	Surface Area (m²)
41	1.3
42	1.3
43	1.3
44	1.4
45	1.4
46	1.4
47	1.4
48	1.4
49	1.5
50	1.5
51	1.5
52	1.5
53	1.5
54	1.6
55	1.6
56	1.6
57	1.6
58	1.6
59	1.7
60	1.7
61	1.7
62	1.7
63	1.7
64	1.7
65	1.8
66	1.8
67	1.8
68	1.8
69	1.8
70	1.9

Body Weight (kg)	Surface Area (m²)
71	1.9
72	1.9
73	1.9
74	1.9
75	1.9
76	2.0
77	2.0
78	2.0
79	2.0
80	2.0
81	2.0
82	2.1
83	2.1
84	2.1
85	2.1
86	2.1
87	2.1
88	2.2
89	2.2
90	2.2

Caution (for children less than 10kg body-weight):

- For children less than 10kg body-weight, dosing by body surface area represents a change in usual clinical practice: This will result in an increase in calculated dose.
- The implications of this change, in clinical practice, are not known in terms of drug toxicity.
- Recommendations:

  Starting doses:

  For infants **less than 6 months** of age: **50%** of calculated dose by body surface area.

  For infants **6 months to 1 year** of age: **75%** of calculated dose by body surface area.

  For infants **over 1 year** of age: **100%** of calculated dose by body surface area.
- These doses may be adjusted according to clinical circumstances.
- Individual investigators (protocols) should have clear recommendations for dosing in infants.

*Acknowledgement: Surface Area of the Human Body. Boyd E. The University of Minnesota Press 1935

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16.11 APPENDIX 11: TOXICITY TIMELINE SCHEME