PROTOCOL TITLE

Adoptive therapy with TCR gene-engineered T cells to treat patients with MAGE-C2-positive melanoma and head and neck cancer

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemene Beoordeling en Registratie
AE	Adverse Event
AR	Adverse Reaction
AT	Adoptive T cell therapy
AZA	5-azacitidine
BORIS	Brother Of the Regulator of Imprinted Sites (= ligand for CTCF)
CA	Competent Authority
CGA	Cancer Germline Antigen
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CRS	Cytokine Release Syndrome
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events (CTCAE)
CTCF	11-zinc finger protein or CCCTC-binding factor (= transcriptional repressor)
CV	Curriculum Vitae
DLT	Dose Limiting Toxicity
DMC	Data Monitoring Committee (a.k.a. Data Safety Monitoring Board (DSMB))
ECOG	Eastern Cooperative Oncology Group
EMC	Erasmus university Medical Center
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
HNSCC	Head and Neck Squamous Cell Carcinoma
GCP	Good Clinical Practice
IB	Investigator's Brochure
IC	Informed Consent
IL-2	Interleukin-2
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
MC2	MAGE-C2
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)
MNC	Mononuclear Cell
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
PBMC	Peripheral Blood Mononuclear Cell
Project team	PI, co-investigator and project leaders

PFS	Progression Free Survival
QP	Qualified Person
RECISTv1.1	Response Evaluation Criteria in Solid Tumors, version 1.1
OOS	Out Of Specification
OS	Overall survival
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics; in Dutch: officiële productinfomatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCR	T Cell Receptor
VP	Valproic Acid
WBP	Personal Data Protection Act; in Dutch: Wet Bescherming Persoonsgegevens
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale

- In patients with advanced melanoma and other tumor types, prior clinical studies have shown efficacy of T lymphocytes directed towards tumor antigens. To this end, tumor reactivity can be imposed by the transfer of T cell receptor (TCR) genes into previously non-reactive T cells.
- MAGE-C2 (MC2) is a Cancer Germline Antigen (CGA) that is highly expressed in melanoma and head and neck squamous cell carcinoma (HNSCC), but not in normal mature tissues. MC2 can evoke clinically effective T cell responses, as demonstrated in previous vaccination studies.
- In this phase I/II study, we will investigate the safety of adoptive T cell therapy (AT) with T cells gene-engineered to express MC2-specific TCRs in patients with advanced melanoma or HNSCC.

Objectives

Phase I

- Primary objectives:
  - to study the safety and feasibility of AT with autologous MC2 TCR T cells, combined with epigenetic drug treatment, in patients with advanced melanoma or HNSCC. Adverse events (AEs) will be documented according to CTCAE version 5.0;
  - to define the maximum tolerated dose (MTD) of MC2 TCR T cells in the combination treatment.
- Secondary and exploratory objectives:
  - to study presence and function of MC2 TCR T cells in peripheral blood samples after treatment;
  - to study the systemic release of inflammatory cytokines after administration of autologous MC2 TCR T cells;
  - to document MC2 expression in tumor tissues (when available) prior to and after epigenetic drug treatment;
  - to document the induction of global DNA hypomethylation and histone acetylation in peripheral blood mononuclear cells (PBMC) by epigenetic drug treatment;
    to study immune parameters, in particular T cell parameters, in blood and tumor tissues (when available) prior to and during treatment.

Phase II

- Primary objectives:
  - to assess the efficacy of AT with autologous MC2 TCR T cells at MTD, combined with epigenetic drug treatment, in patients with advanced melanoma or HNSCC;
  - tumor response will be evaluated using RECIST v1.1.[1] Response is defined as complete remission (CR) and partial response (PR) according to RECIST v1.1.
- Secondary objectives:
  - to determine the following outcomes in patients treated with MC2 TCR T cells at MTD:
    - progression free survival;

  - duration of response;
  - overall survival.
- Exploratory objectives:
  - to study presence and function of MC2 TCR T cells in peripheral blood samples after treatment;
  - to study the systemic release of inflammatory cytokines after administration of autologous MC2 TCR T cells;
  - to document MC2 expression in tumor tissues (when available) prior to and after epigenetic drug treatment;
  - to document the induction of global DNA hypomethylation and histone acetylation in peripheral blood mononuclear cells (PBMC) by epigenetic drug treatment;
  - to study immune parameters, in particular T cell parameters, in blood and tumor tissues (when available) prior to and during treatment.

Study design

- This is a single institution phase I/II trial consisting of an accelerated titration phase I design and a subsequent single arm (2-stage) phase II study. In the phase I part, the recommended T cell number for phase II will be determined.
- Eighteen patients with advanced melanoma or HNSCC, a HLA-A2 genotype and MC2-positive tumors will be included in the study.
- Screening will consist of genotyping for HLA-A2 and MC2 immunohistochemistry on tumor tissue.
- Prior to T cell transfer (day 0), patients will be treated with the epigenetic drugs valproic acid (VP, dose 50 mg/kg/d, 7d; days -9 to day -3) and 5-azacitidine (AZA, dose 75mg/m^2/d, 7d; days -9 to day -3).
- Phase I: patients will be treated with one single intravenous administration of TCR T cells at 5 different escalated doses of $5x10^7$, $5x10^8$, $5x10^9$,$1.0x10^{10}$, and the total number of cultured TCR T cells (i.e. usually 1.0 – 5.0 $x10^{10}$ TCR T cells). MC2 TCR Tcell infusions will be supported by low dose of IL-2 administrations (s.c. $5x10^5$ IU/m^2 2qd for 5 days).
- In case of dose limiting toxicities (DLT) in the accelerated titration phase, the phase I part will continue in a classical 3+3 design and dose-escalation will be semi-logaritmic.
- In case 1 (or 3) patient(s) has(ve) been treated in a particular dose level, but not before 3 weeks after treatment of the (3^{rd}) patient, AEs will be evaluated and the project team (PI, co-investigator and project leaders) will decide whether the trial will continue to the next dose level in phase I or will continue using the current dose level in a classical 3+3 design.
- If DLT is observed in 2 out of 6 patients (3+3 extended cohort), the previous (semi-logaritmic) dose level will be the maximum tolerated dose (MTD) (see § 4.4).
- Phase II: patients will be treated with TCR T cells at the MTD. In case no DLTs were observed, patients will be treated with the total number of cultured TCR T cells, which is usually 1.0 – 5.0 $x10^{10}$ TCR T cells.

- In both phase I and II, peripheral persistence, differentiation state and tumor recognition of peripheral and intra-tumoral TCR T cells will be evaluated, as well as additional immune and T cell parameters in blood and tumor tissue.

Study population

Patients with unresectable stage IIIc and IV melanoma (including unknown primary, mucosal and uveal melanoma), and unresectable recurrent/metastatic (R/M) HNSCC who are ≥18 years of age are eligible. Melanoma patients must have progressive disease after standard treatment including for instance immune checkpoint inhibition, BRAF/MEK-inhibition and chemotherapy. R/M HNSCC patients who are ineligible for or unwilling to get platinum-based chemotherapy or for whom no standard treatment is available are eligible. Only patients with a HLA-A2 genotype and MC2-positive tumors will be included.

Intervention

Autologous T cells, obtained by leukapheresis, will be transduced with a retroviral vector encoding the MC2 TCR. Subsequently, these TCR T cells will be expanded *ex vivo* in the presence of defined cytokines. Following pre-treatment with epigenetic drugs, TCR T cells will administered intravenously with support of low-dose IL-2. For safety measures, AEs will be documented according to CTCAE 5.0. Tumor response will be evaluated using RECIST v1.1. Tumor biopsies will be taken before treatment (to assess MC2 expression) and where possible at 3 weeks after MC2 TCR T cell infusion and at progressive disease to evaluate therapy induced changes of the tumor microenvironment. An optional tumor biopsy may be taken after epigenetic pre-treatment and prior to MC2 TCR T cell infusion to document the in vivo upregulation of the MC2 expression.

Main study parameters/endpoints:

Primary endpoints

Phase I

- AEs according to CTCAE 5.0;
- recommended Phase II dose;
- feasibility to deliver this sequence of treatment.

Phase II

- objective response rate according to RECIST v1.1;
- PFS;
- OS.

Secondary and exploratory endpoints

Phase I & II

- persistence and function of MC2-specific T cells in peripheral blood;
- systemic release of inflammatory cytokines after administration of autologous MC2 TCR T cells;
- MC2 expression in tumor tissues prior to and after epigenetic drug treatment;

- global DNA hypomethylation and histone acetylation in PBMCs after epigenetic treatment and administration of autologous MC2 TCR T cells;
- immune parameters, in particular T cell parameters, in blood and tumor tissues (when available) prior to and during treatment.

Nature and extent of burden risks associated with participation, benefit group relatedness

For patients included in this phase I/II trial, no standard therapies are available in the Netherlands. Previous trials have shown efficacy of AT in several patient populations.

In patients with metastatic melanoma or synovial carcinoma, AT with a CGA NY-ESO TCR T cells has shown very limited AEs and significant objective clinical responses of 55 and 61%, respectively.[2, 3] In patients with multiple myeloma, infusion of NY-ESO TCR T cells 2 days after an autologous stem cell transplant (preceded by Melphalan) resulted in a clinical response of 80%, thereby inducing various serious AEs (SAEs), which were reversible.[4]

In patients with melanoma, AT using autologous TCR T cells with affinity enhanced TCRs for MAGE-A3 has shown clinical responses, but also fatal on- and off target toxicities as a result of either targeting a common epitope of the MAGE-A antigen superfamily or a TCR with a non-restricted binding motif.[5, 6] In the current study, these (S)AEs are not expected as the MC2-specific TCR has a restricted binding motif without enhanced affinity. Nevertheless, in case patients may experience a cytokine release syndrome (CRS) with severe symptoms in the current study, patients will be treated with high dose corticosteroids, the anti-IL6R antibody tocilizumab [7], and the anti-CD52 antibody alemtuzumab, depending on the severity of the symptoms. In addition, the doses of the administered epigenetic drugs are well tolerated and are only associated with mild transient leucopenia and neutropenic fever.[8] Toxicities of epigenetic drugs and IL-2 are expected to be mild and managible by standard supportive care. In this patient population, the potential burden and AEs are justified by the substantial chance of (durable) tumor responses as a result of the proposed treatment.

The study proposal contains the following unique elements (see Figure 1):

1. targeting MC2 by TCR T cells;
2. a new T cell processing method to generate young T cells;
3. pretreatment with epigenetic drugs to increase MC2 expression in the tumor;
4. no chemotherapy prior to T cell infusion, which are usually administered in other trials to support modified T-cells, but which also substantially increase toxicity.

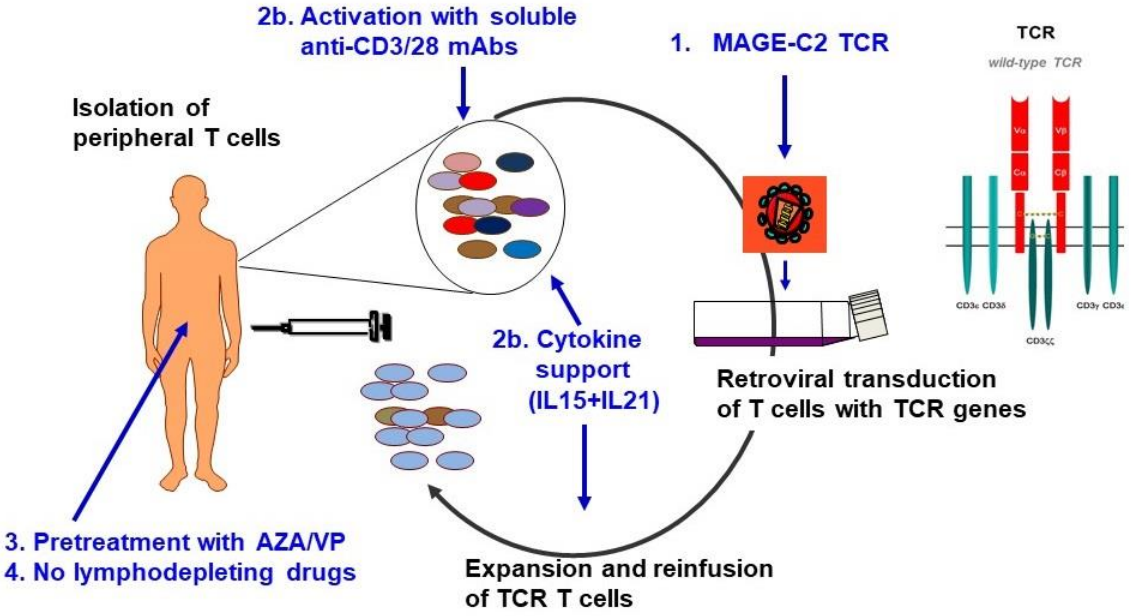

Figure 1: Adoptive T cell therapy with MC2 TCR T cells - overview of procedures and treatment.

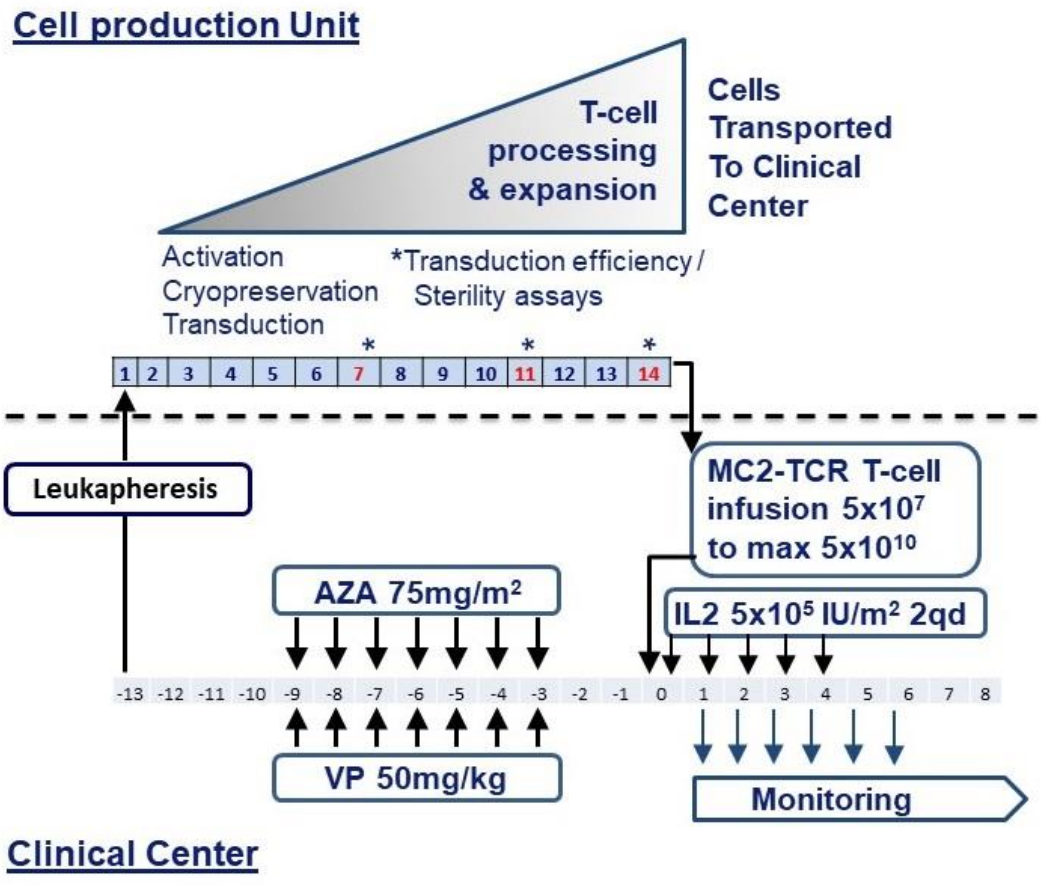

Figure 2: Adoptive T cell therapy with MC2 TCR T cells - schedule of laboratory and clinical procedures. For monitoring schedule, see Table 1b

1. INTRODUCTION AND RATIONALE

1.1 Clinical background

In 2018, 7000 novel cases of melanoma were diagnosed in The Netherlands and incidence rates are still increasing.[9] Until 2012, the OS of patients with unresectable stage IIIc and metastatic melanoma was only 6-10 months.[10, 11] The introduction of targeted therapy (BRAF and MEK inhibitors) and immune checkpoint inhibitors (anti-CTLA-4 and anti-PD-1 antibodies) has significantly improved the perspectives and survival of these patients.[12-20]. Nevertheless, approximately 50% of patients with cutaneous and mucosal melanoma does not have (long-term) benefit and eventually experience progressive disease.[11] Although novel therapies have proven efficacy in cutaneous and mucosal melanoma, there is still a large fraction of patients that show non-durable responses.[11] On top of that, systemic therapy is still disappointing for metastatic uveal melanoma; in fact, standard systemic therapy is lacking for patients with metastatic uveal melanoma and their OS is poor with 8-13 months.[21, 22] Each year over 800 patients die of melanoma in The Netherlands.[11]

In 2018, 3100 novel cases of HNSCC were diagnosed in The Netherlands (NKR) and incidence rates are gradually increasing.[23] After initial diagnosis and multidisciplinary treatment, a substantial proportion of patients are diagnosed with recurrent/metastatic (R/M) HNSCC. For patients with R/M HNSCC cetuximab added to platinum-5-fluorouracil is the only approved treatment with significant overall survival benefit.[24]However, the life expectancy still is less than 1 year and the toxicity of this regimen is considerable. In patients with R/M HNSCC, PD-1 blockade is a promising new treatment option. In a randomized phase III trial, treatment with nivolumab resulted in a longer survival than treatment with chemotherapy.[25] Pembrolizumab as first-line treatment significantly improved overall survival over platinum/5-fluorouracil/cetuximab in patients with R/M SCCHN with a PD-L1 combine positive score (CPS) ≥20.[26] Despite the therapeutic benefit of these new treatments, the OS of patients with R/M HNSCC remains poor.

In conclusion, there is a need to develop new treatment strategies for a substantial number of patients with advanced and metastatic melanoma and HNSCC. In the current phase I/II trial, adoptive T cell transfer targeting MAGE-C2 (MC2), a tumor specific target in melanoma and HNSCC, will be evaluated.

1.2 Adoptive T cell transfer (AT): previous clinical successes

1.2.1 AT using tumor-infiltrating Lymphocytes (TILs)

There is substantial clinical evidence that AT leads to objective responses in melanoma patients. In the past 15 years, several clinical trials have demonstrated that AT with TILs is a safe and effective strategy for patients with metastatic melanoma.[27-32] These treatments yielded impressive objective response rates of 50% with a five year OS rate of ~20%.[32, 33] Recently, tumor-reactive TILs also have been successfully grown from other tumor types, including renal cell, ovarian, breast, bile duct and cervical cancer.[34-37] In patients with bile duct and cervical cancer, TIL therapy was also

effective and demonstrated control of tumor growth.[38] [34] To date, generation of TILs from solid tumors is successful in only about half of the patients.[32]

1.2.2 AT using chimeric antigen receptor (CAR) T cells

A more universal strategy to generate tumor-specific T cells for clinical application is based on the transfer of genes encoding a chimeric antigen receptor (CAR) or a T cell receptor (TCR). CARs are antibody-based receptors and recognize extracellular antigens in a major histocompatibility (MHC) unrestricted fashion (representing about 25% of all antigens), generally with high binding strength.

After breakthrough studies using CD19 CAR T cells for refractory B cell malignancies [39, 40], the number of clinical applications for CAR-T cells are rapidly expanding.[41-44] Over the last 2 years, 2 products of CD19-CAR T cells have been granted FDA approval for various B cell malignancies, i.e., tisagenlecleucel (KYMRIAH®, Novartis) and axicabtagene ciloleucel (YESCARTA®, Kite Pharmaceuticals).[44] Until now, tumor responses with CAR T cells in patients with B cell malignancies have not yet been consistently reproduced for patients with solid tumors (see § 1.2.3). However, CAR-T cell development for patients with solid tumors is still in its early stages with an extending number of ongoing and expected clinical trials (www.clinicaltrials.gov).

1.2.3 AT with CAR-T cells: a pioneering study at Erasmus MC

Between 2003-2010, we have treated 12 patients with autologous T cells redirected against renal cell carcinoma (RCC) via a CAR specific for carboxy-anhydrase IX (CAIX) that is over-expressed by RCC. Per original protocol 3 patients, and per amendment another 5 patients were treated with $1x10^9$ CAIX CAR-T cells and low dose IL-2. Four out of these 8 patients presented with severe, but transient liver toxicities, which were most likely due to CAIX expression on the surface of epithelial cells lining the bile ducts in the liver (albeit at a low level) [45, 46]. A second amendment enabled pre-treatment with 5 mg of the humanized anti-CAIX mAb. Based on the favorable kinetics of this antibody, pre-infusion was expected to cover CAIX expressed by normal tissue, thereby leaving CAIX expression in RCC targetable for T cell treatment [46]. With this adapted regimen consisting of pre-infusion with humanized anti-CAIX mAb, treatment with CAR-T cells ($1x10^9$ - $2x10^9$) and low dose IL-2, patients (n=4) did not experience any adverse events.[45, 46] However, CAR-T cells did not persist in the circulation for more than 30 days following infusion. T cell persistence and anti-tumor responses may have been limited because of the low T cell doses as well as the mature differentiated status of the infused T cells.[47] As several new treatment options for RCC were introduced in the clinic from 2005 onwards, patient accrual was hindered and this T cell study was prematurely discontinued in 2010.

1.3 AT with TCR T cells

1.3.1 Clinical experience and challenges

Whereas CARs recognize extracellular antigens in a MHC unrestricted fashion (representing about 25% of all antigens), with high binding strength, TCRs recognize both extra- and intracellular antigens

(representing all antigens) with low-to-medium binding strength. As a result, TCRs have a wider breadth of targetable antigens. To date, a limited number of clinical trials has applied TCR T cells for the treatment of solid tumors (see [48, 49], and about a few dozen of these trials are ongoing (www.clinicaltrials.gov).
In previous clinical trials, TCRs were usually restricted to the HLA-A1 or A2 genotype and directed against differentiation antigens (i.e. MART-1, gp100, CEA, and p53), Cancer Germline Antigens (i.e. MAGE-A3 and NY-ESO-1), and viral antigens. Collectively, these trials demonstrated responses in 12 to 80% of patients with metastatic melanoma, colorectal carcinoma, synovial sarcoma, and multiple myeloma.[2-4] However, treatment-related AEs became evident from studies with TCRs, in particular for TCRs with high-affinity, directed against antigens with over-expression in both tumors and healthy tissue.[5, 50-52]. Two previous clinical studies applied TCRs directed against MAGE-A3 (2 different epitopes) and reported fatal neurological and cardiovascular AEs [5, 52]. These AEs were most likely caused by the use of affinity-enhanced TCRs that recognize a shared or highly similar epitope compared to the cognate epitope. As previously mentioned in § 1.2.3, the above-mentioned AEs require careful selection and preclinical assessment of T cell target antigens.[53, 54] Besides limiting toxicity, durable tumor responses need to be achieved upon treatment with TCR T cells.[48, 55] In previous studies, short duration of tumor responses was often associated with a short persistence of TCR T cells in blood.[56, 57]

Collectively, findings in previous clinical trials demonstrate that TCR T cell therapy is promising and indicate that further development of TCR T cell therapy should focus on (see § 1.3.2 for more detail):

- Selection and validation of effective and safe target antigens for T cells
- Strategies to enhance TCR T cell persistence and anti-tumor responses

1.3.2 Unique elements of current Erasmus MC trial with TCR T cells

1.3.2.1 MAGE-C2 is a 'safe' target antigen

MAGE-C2 (MC2) is a tumor specific target in melanoma and HNSCC. We have developed T cells which are gene-engineered to express MC2-specific TCRs. For treatment with these MC2 TCR T cells, risk of severe AEs is considered negligible since we do not use an affinity-enhanced TCR, as the TCR is derived from a patient without signs of toxicity. In addition, on- or off-target recognition was not observed in our preclinical studies (see below for details).

We have selected the HLA-A2-restricted $MC2_{336-344}$ epitope ALKVDVEERV as a target for AT for the following reasons:

- MC2 protein is expressed in 40% of metastatic melanomas and 20% of HNSCCs and its expression is associated with poor clinical outcome in these tumor types (http://www.cta.lncc.br)[58-60];
- MC2 is not expressed in healthy tissues, with the exception of gonads. Nevertheless, the gonads are excluded for on-target recognition, as the gonads are immune-privileged and have no MHC

expression. This is supported by the observation that the presence of high numbers of MC2 T cells (in a vaccinated patient) was not associated with AEs [61, 62];

- MC2 protein actively contributes to the development of malignancies by facilitating angiogenesis and migration of tumor cells, and suppressing p53-dependent apoptosis and epithelial-to-mesenchymal transition (EMT) [63];
- MC2 protein is immunogenic and is associated with clinical responses. In at least 2 melanoma patients several MAGE-C2 specific T cell clones were identified that showed increased frequencies in blood and regressing tumor lesions following vaccination.[61, 64]
- MC2 ALK peptide is significantly bound by HLA-A2, a property that may facilitate cross-presentation and contribute to effective T cell responses [65];
- MC2 ALK peptide is, in contrast to other antigenic peptides, favored by immune proteasomes [66];
- MC2 ALK peptide is an immunogenic epitope as demonstrated by its ability to induce a T cell response with durable tumor response and without AEs [61, 62].

In melanoma patients with durable tumor responses following MAGE-A3 vaccination, T cell clones targeting MC2 epitopes have been identified. From melanoma patient EB81 with a > 3 yrs complete response, amongst others, the T cell clone EB81-CTL16 was isolated, targeting the $MC2_{336-344}$ epitope ALKVDVEERV.[61, 62] CTL EB81-CTL16 demonstrated a 1000-fold increase in numbers in a regressing cutaneous metastasis and represented about 10% of T cells present in an invaded lymph node. These observations strongly suggested a role of this T cell clone in the observed tumor response. In the same patient, other T cell clones, specific for the same and other MC2 epitopes, also showed increased frequencies in blood and a regressing metastasis.[61, 62] In another melanoma patient (LB2586) with tumor response after MAGE-A vaccination, the most frequent anti-tumor T cell clone was again directed against a MC2 epitope.[64]

The MC2 TCRαβ genes derived from EB81-CTL16 (provided by our collaborator: prof. Pierre Coulie, Univ Louvain, Brussels, Belgium) were sequence characterized (Vα3.01/Jα35.01/Cα; Vβ28.01/Jβ1-6.01/Cβ1), codon optimized (GeneArt, Regensburg, Germany) and cloned into the pMP71 vector separated by an optimized T2A ribosome skipping sequence. When introduced into primary human T cells, the MC2 TCRαβ genes were functionally expressed. Our in vitro studies revealed that the MC2 TCR16 displays a low-to-intermediate ligand-binding affinity (ED50=0.75 nM [54, 67]) and a dependency for the CD8α co-receptor. Furthermore, MC2 TCR16 T cells, when incubated with native MC2/A2+ tumor cells, displayed antigen-specific cytolysis and INFγ production.[54, 67] TCR expression and function could be maintained in cells kept in culture for longer than 3 weeks.

Besides efficacy, we have also evaluated the toxicity risks for the MC2 target antigen as well as its corresponding TCR16. First, expression of MC2 antigen in healthy human tissues was assessed. Quantitative PCR analysis on a cDNA library of 48 healthy human tissues demonstrated absence of MC2 mRNA in healthy tissues. In addition, using an MC2-specific antibody, we confirmed the presence of MC2 protein in testis and melanoma as well as its absence in multiple healthy tissues,

such as brain, heart, intestine, and lung.[54] For TCR16, the recognition motif toward its cognate peptide and the cross-reactivity toward non-cognate peptides were evaluated. These analyses showed a highly stringent TCR recognition motif towards the cognate antigen (7 of 9 amino acids: xLKDVEERx) with cross-reactivity limited to MAGE-C1, i.e. a related CGA without expression in healthy non-gonadal tissues. Collectively, these analyses showed absent MC2 expression in somatic healthy tissues, pointing towards a beneficial safety profile for MC2 TCR16 (further MC2 TCR).[54]

1.3.2.2 Therapeutic T cells have a 'young' phenotype and warrant improved peripheral persistence

Furthermore, we have defined a T cell activation and processing method that includes T cell activation with soluble anti-CD3/CD28 mAbs in the presence of both IL-15 and IL-21 prior to TCR gene transfer and which results in enhanced proportions of T cells with a 'young' phenotype.[68-70] In more detail, T cells generated according to this processing method demonstrated enhanced binding of pMHC [HLA-A2/MC2$_{ALK}$], enhanced frequency of CD27+, CD62L+, CD45RA+ T cells, and enhanced *in vitro* MC2-specific T cell function. This T cell processing method has been included in the existing GMP protocol [71, 72] to generate MC2 TCR T cells for clinical application.

1.3.2.3 Combination treatment with epigenetic drugs

To enhance the efficacy of MC2 TCR T cells, epigenetic treatment is promising, as epigenetic treatment can induce increased *de novo* and sustained expression of CGAs, such as MAGE-A1, -A3, -A4, NY-ESO-1 in tumor cell lines *ex vivo* and in human tumors *in vivo*.[73, 74] Effective and safe clinical regimes with the epigenetic drugs valproic acid (VP) and 5-azacitidine (AZA) have been developed for the treatment of patients with advanced solid tumors, acute myeloid leukemia (AML) and high risk myelodysplasia syndromes (MDS).[8, 75, 76] Yet the AZA+VP treatment regimen come with AEs in more than 10% of patients, in particular (febrile) neutropenia, leukopenia and nausea/vomiting, for which effective anti-emetic prophylaxis can be applied.[8, 76, 77] In AML patients, AZA+VP treatment was able to induce an anti-MAGE T cell response.[75] Furthermore, DNA hypo-methylation is a beneficial prognostic marker in patients with stage IIIC melanoma.[78]

We have extended these observations and demonstrated that pre-treatment with AZA+VP significantly increased MC2 gene expression in melanoma and HNSCC tumor cell lines and enhanced their ability to elicit an IFNγ response by MC2 TCR T cells. After treatment with AZA+VP, we showed selective induction of gene and protein expression of MC2 in tumor cells, but not in normal cells.[79] Literature studies show that de-repressed expression in CGAs, including NY-ESO1, MAGE-A1 and MAGE-A3, the is related to epigenetic changes, one of them being a shift from the transcription factor CTCF to BORIS. Normal cells do not express BORIS and potentially have additional multiple silencing mechanisms still intact, making them less sensitive to epigenetic drugs than tumor cells.[80-83]

The proposed doses and schedule of the two epigenetic drugs in this protocol have been assessed and applied in several clinical studies [8, 76, 84-86] and consist of subcutaneous administration of

AZA at an dose of 75mg/m^2/d;q7d and oral administration of VP at a dose of 50 mg/kg/d;q7d. With respect to pharmacokinetics, AZA reaches maximal concentration in blood (Cmax) at 30 min after subcutaneous administration with an elimination half-life of 41 min and sub-therapeutic levels at approximately 3h (= 4-5 x half-life). After oral administration, VP reaches Cmax in blood after 1-6 hours with an elimination half-life of 10-15 hours and sub-therapeutic levels at approximately 2 days. In PBMCs, this combination of AZA and VP has shown to induce global DNA hypomethylation and histone acetylation.[8]

1.3.2.4 No pre-treatment with chemotherapy

For TIL-based therapy, pre-treatment with non-myeloablative chemotherapy and (high dose) IL-2 after T cell infusions were required to achieve efficacy.[28, 33, 87] This strategy has been incorporated in most recent trials with gene-engineered T cells, despite the fact that its benefit was formally not investigated. Since chemotherapy increased the risk of AEs we consider it worthwhile to revisit the need for this pre-treatment, which is supported by two clinical reports. First, Butler and co-workers [88] showed that MART1-specific T cells, generated using co-stimulatory artificial antigen presenting cells (aAPC), survived for prolonged periods in advanced stage melanoma patients without previous chemotherapy or high-dose IL-2 treatment. We argue that a mild myelosuppression/leukopenia induced by AZA and VP [8, 77, 89] as well as the generation of young T cells [68] are expected to eliminate the need for prior chemotherapeutic lymphodepletion.

1.3.2.5 Low-dose Interleukin-2

IL-2 is a T cell growth factor that supports the proliferation, function and survival of T cells both in vitro and in vivo. Initially, the anti-tumor activity of IL-2 was investigated in trials applying high-dose IL-2 (15-24 x 10^6 IU/m^2/day), delivered systemically, either as a bolus injection or as a 24 hr continuous infusion. However, the high-dose systemic IL-2 was associated with severe toxicities.[90-94] Subsequent studies showed that treatment with low-dose IL-2 administered subcutaneously (s.c.) was well tolerated with only mild toxicity (grade I-II) ([95] and systematic review [96]) and that a low-dose of IL-2 was sufficient to achieve immuno-modulatory effects such as pre-activation of T-cells.[97, 98]

In this study we apply a low-dose of 1x10^6 IU IL-2/m^2 daily for a period of 5 days following the MC2 TCR T cell administration. In our former CAIX CAR T cell trial (P00.0040C) we have applied the same IL-2 dose for up to 20 days, without noticeable IL-2 related toxicities ([46]). In addition, similar IL-2 dose and schedules are presently applied in several clinical TCR T cell studies (see clinicaltrial.gov: NCT03462316 and NCFT03029273 (NY-ESO-1- TCR T cells); NCT02550535 (WT1 TCR T cells) NCT03686124 and NCT03247309 (ACTengine T cells).

1.3.4 Safety considerations of Erasmus MC AT trial with TCR T cells

1.3.4.1 No expected on- and off-target toxicity

On-target toxicity is not expected as MC2 is *not* expressed by somatic human cells in healthy individuals and cancer patients.[54] MC2 is only expressed by germline cells in the gonads, which are immune privileged organs. Treatment with AZA+VP has been demonstrated to increase CGA expression in tumors [79], yet no signs have been reported of auto-immunity towards gonads following patient treatment with these drugs. Therefore, AZA+VP treatment is highly unlikely to induce gonadal recognition by MC2 TCR T cells. In addition, the MC2 TCR was isolated from a melanoma patient who showed a complete remission following a MAGE-A1 and MAGE-A3 vaccination regimen, without any clinical toxicity.

Also, off-target toxicity is not expected as TCR16, recognizing the ALK epitope in the context of HLA-A2, has a very restricted recognition motif towards the ALK epitope, and TCR cross-reactivity was only found towards MAGE-C1, a related CGA that is not expressed in healthy non-gonadal tissues.[54] In AT with TCR T cells directed against the melanocyte differentiation antigen MART-1, melanocyte destruction in skin, eye and ear (leading to vitiligo, uveitis and hearing loss) have been reported([50, 51] NKI: NL.37327.000.11). No such toxicities are expected for TCR16 T cells since MC2 is not expressed in normal melanocytes or other cells derived from normal tissues.[54]

1.3.4.2 No expected toxicity associated with retroviral integrations

Retroviral transductions may potentially lead to malignant transformation when the therapeutic gene integrates in/nearby oncogenes. This genotoxicity was observed in a gene therapy trial of XSCID with the intent to gene correct hematopoietic stem cells, where 5 children developed leukemia upon retroviral integration in the LMO2 oncogene.[99, 100] However, the risk of transforming events upon infusion of TCR T cells is likely to be negligible when compared to the X-SCID trial. To date, dozens of clinical AT studies with retro- or lentiviral gene-modified T lymphocytes have been performed with no reports regarding toxic viral integrations. The recently reported leukemia in a patient treated with CD19 CAR T cells appeared to be derived from a transduced leukemic B cell that was present in the leukapheresis product at start of treatment but not from treatment with CAR T cells.[101]

1.3.4.3 No expected toxicity related to activated and expanded T-cells in vivo

In several studies using TILs or CARs, the AT of T cells has been complicated by the cytokine release syndrome (CRS) with high plasma levels of cytokines such as IL-6 and
IFN-γ. In some patients, CRS can induce high fevers, rigors, hypotension, and heart failure.[39, 42, 102] In case of severe deterioration of the patient's condition, escape medication consisting of high dose corticosteroids in conjunction with anti-CD52 antibody (alemtuzumab) may be given.[103] Severe and potentially life-threatening CRS can effectively be treated with the anti-IL-6 receptor antibody tocilizumab.[104, 105] Although we formally cannot exclude the chance that CRS may occur, CRS has

mainly been described for CAR-T cells in patients with high tumor load in the blood compartment. Therefore, CRS is not or to a lesser extent expected in patients with solid tumors.

1.3.4.4 No severe toxicity expected for pre-treatment with AZA+VP

In clinical practice, AZA is administered for the treatment of several hematological malignancies and is associated with AEs in more than 10% of patients, in particular (febrile) neutropenia and leukopenia.[8, 76, 77]. In clinical practice, VP is primarily prescribed for the treatment of epilepsy, bipolar disorder, and prevention of migraine headaches. During VP treatment, toxicities are limited, and hematological toxicities are rare.

Hematological toxicity is the most frequently reported severe toxicity for the combination AZA and VP. In a phase I study investigating the combination of VP (maximum dose 60 mg/kg/d for 28 days) and AZA, the maximum tolerated dose for AZA was 75mg/m^2/d for 10 days.[8] At that dose, which was expanded, 3 of 16 patients (18%) showed dose-limiting grade 3-4 toxicity (neutropenic fever). Other toxicities were grades 1 and 2 somnolence (six patients), tremor (six patients), hypomagnesemia (one patient), anemia (two patients), and vomiting (one patient). In the current study, VP will be administered at a lower dose (50 mg/kg/d) and a shorter treatment duration (7d), whereas the treatment duration of AZA is also shorter (7d). Therefore, the toxicity of the combination of AZA and VPis expected to be limited in the current study.

In the current study, MC2 TCR T cells will be infused 2 days after the last AZA+VP dose. Regarding the short half-life of AZA, no direct effect of peripheral AZA can be expected on infused T cells. Of note, Cruz and colleagues [73] showed that *in vitro* treatment of T cells with AZA did neither compromise T cell phenotype nor function. The waning blood levels of VP in absence of AZA are also not expected to affect the T cells.

1.3.4.5 No severe toxicity expected for low-dose IL-2

The applied dose of IL-2 is chosen such that only minor toxicities can be expected. From the systematic review [96] is concluded that administration of low-dose rIL-2 can be considered safe for clinical and experimental use in humans since an overall low rate of severe adverse events was observed, limited to thrombocytopenia. Most frequently local reactions at the injection sites were reported and other (grade I-II AE) reactions included, influenza-like syndrome, headache and dyspnea.

2. OBJECTIVES

2.1 Phase I

2.1.1 Primary objectives

- to study the safety and feasibility of AT with autologous MC2 TCR T cells, combined with epigenetic drug treatment, in patients with advanced melanoma or HNSCC. AEs will be documented according to CTCAE version 5.0;

- to determine the recommended phase II dose, based on the MTD of MC2 TCR T cells in the combination treatment.

2.1.2 Secondary and exploratory objectives

- to study presence and function of MC2 TCR T cells in peripheral blood samples after treatment.
- to study systemic release of inflammatory cytokines after administration of autologous MC2 TCR T cells;
- to document MC2 expression in tumor tissues (when available) prior to and after epigenetic drug treatment;
- to document the induction of global DNA hypomethylation and histone acetylation in PBMCs by the epigenetic drug treatment;
- to study immune parameters, in particular T cell parameters, in blood and tumor tissues (when available) prior to and during treatment.

2.2 Phase II

2.2.1 Primary objective

- to study the efficacy of AT with autologous MC2 TCR T cells at MTD, combined with epigenetic drug treatment, in patients with advanced melanoma or HNSCC.

2.2.2 Secondary objectives

To determine the following outcomes in patients treated with MAGE-C2 TCR T cells at MTD:

- progression free survival;
- duration of response;
- overall survival.

2.2.3 Explorative objectives

- to study persistence of MC2 TCR T cells, their differentiation state and ability to recognize tumor cells at regular intervals (see § 7.3.3) following start of T cell treatment.
- to document MC2 expression in tumor tissues (when available) prior to and after epigenetic drug treatment.
- to document the induction of global DNA hypomethylation and histone acetylation in PBMCs.
- to assess presence of immune and T cell parameters in blood, and micro-environment of tumor tissues using advanced flow cytometry and in situ stainings.
- to assess response rate according to immune related Response Criteria (irRC).[106]

3. STUDY DESIGN

3.1 Description of the study design

In this open label non-randomized single institution phase I/II trial, the safety and efficacy of epigenetic drugs followed by transfer of TCR T cells will be evaluated in patients with advanced melanoma or HNSCC.

This study consists of two parts:

- phase I: dose escalating;
- phase II: expansion.

Patients will be screened for HLA-A2 by genotyping and tumors will be screened for MC2 by immunohistochemistry. Eligible patients will undergo leukapheresis to retrieve sufficient T cells which will be MC2 TCR gene-engineered and expanded in the laboratory as described (see IMPD). Patients will be treated with an epigenetic drug regimen consisting of AZA (75 mg/m^2/day s.c. x 7 days), and VP (50 mg/kg/day p.o. x7 days). Two days following this regimen, one single intravenous adoptive transfer of autologous MC2 TCR T cells will be administered, starting with the first dose level. The treatment schedule is depicted in Figure 2.

3.1.1 Phase 1: dose escalating phase

Patients will be treated according to an accelerated titration phase I trial design.[107] This design incorporates a rapid dose escalation, reduces the number of patients who are undertreated, facilitates completion of the phase I trial, and provides significant information on tolerability and the establishment of a recommended T-cell number for further testing in the phase II study.

Patients will be treated in subsequent cohorts of 1 patient according to the following dose-escalation scheme:

dose level 1: 5.0x10^7 MC2 TCR T cells;
dose level 2: 5.0x10^8 MC2 TCR T cells;
dose level 3: 5.0x10^9 MC2 TCR T cells;
dose level 4: 1.0x10^{10} MC2 TCR T cells;
dose level 5: the total number of cultured cells, being typically 1.0 – 5.0 x10^{10} MC2 TCR T cells.

After 1 (3) patient(s) has/have been treated in each dose level, but not before 3 weeks after the patient has been infused with TCR T cells, the project team (PI and co-investigators) will decide, based the observed AEs, whether the trial will be continued at the next dose level or will continue in the current dose level (see § 4.4). From the moment onwards that AEs are observed, next dose escalations will be semi-logarithmic.
DLT is defined as any CTCAE grade 4 and non-manageable grade 3 toxicity within a 21 day DLT period.

Clinical evaluation for toxicity and efficacy will consist of comprehensive clinical follow-up, laboratory tests and conventional imaging (e.g. computed tomography and magnetic resonance imaging). At 6 and 12 weeks and every 3 months thereafter response evaluation will be performed. See § 7.3 for scheduled visits and specific procedures.

For translational analyses, peripheral blood samples and tumor biopsies will be collected as well (see Figure 3).

3.1.3 Phase II: expansion phase

A Simon-2 stage single arm phase II design will be applied.[108] The study will continue the first stage at the recommended TCR T cell dose number defined in phase I until a total of 6 patients have been enrolled and treated. Clinical responses and safety data of these first 6 patients will be evaluated by the DMC. If no clinical responses will be observed, the trial will be discontinued and the conclusion will be that in the applied setting the TCR lacks efficiency; otherwise, the trial will continue to its second stage.

In the second stage, overall 12 patients will be enrolled (including the first stage): if the total number of responses for the two stages combined is less than 3, the trial will be discontinued as soon this is evident and the conclusion will be that in the applied setting the TCR lacks efficiency. Clinical responses and safety data of the patients will be evaluated by the DMC.

4. STUDY POPULATION

4.1 Population

In this trial two groups of patients will be enrolled:

1) patients with inoperable stage IIIc/IV melanoma with progressive disease after standard therapy;
2) patients with R/M HNSCC who are ineligible for or unwilling to get platinum-based chemotherapy or for whom no standard treatment is available.

4.2 Inclusion criteria

Subjects eligible for inclusion in this study have to meet all of the following criteria:

- written informed consent must be obtained prior to any screening procedures.
- male or female must be ≥ 18 years of age at time of providing informed consent.
- one of the following three malignancies:
    1. previously treated for unresectable or metastatic cutaneous or mucosal melanoma for whom no standard treatment is available (anymore);
    2. metastatic uveal melanoma, progressing after standard of care therapy, if available;
    3. R/M HNSCC for whom no standard treatment is available anymore;

- patients must be HLA-A2*0201 positive;
- primary tumor and/or metastasis (archival or fresh biopsy) is positive for MC2 (> 5% of tumor cells) according to immunohistochemistry;
- measurable disease according to RECIST v1.1;
- at least one lesion, suitable for sequential mandatory tumor biopsies;
- ECOG performance status of 0 or 1. Life expectancy ≥ 12 weeks (see Appendix 1);
- patients with melanoma must have had objective evidence of disease progression while on or after standard systemic therapy. The last dose of prior therapy (e.g. anti-PD-1, chemotherapy) must have been received more than 4 weeks prior to the start of study treatment. For melanoma patients who are treated with BRAF- and MEK inhibitors, an interval of 2 weeks between discontinuation of BRAF- and MEK inhibition and start of study treatment is sufficient;
- patients with R/M HNSCC must have had objective evidence of disease progression and are ineligible for or unwilling to get platinum-based chemotherapy or for whom no standard treatment is available;
- patients of both genders must be willing to practice a highly effective method of birth control during treatment and for four months after receiving the preparative regimen;

Patients must meet the following laboratory values at the screening visit in the absence of growth factors and/or transfusion support:

- hematology:
  - absolute neutrophil count greater than $1.5x10^9$/L;
  - platelet count greater than $75x10^9$/L;
  - hemoglobin greater than 5 mmol/L or 8.0 in g/dl;
- chemistry:
  - serum ALAT/ASAT less than 3 times the upper limit of normal (ULN), unless patients have liver metastases (< 5 times ULN);
  - serum creatinine < 1.5 ULN;
  - total bilirubin less than or equal to 20 micromol/L, except in patients with Gilbert's Syndrome who must have a total bilirubin less than 50 micromol/L;
- serology:
  - seronegative for HIV antibody;
  - seronegative for hepatitis B antigen, and hepatitis C antibody;
  - seronegative for lues.

4.3 Exclusion criteria

Subjects who meet any of the following criteria will be excluded from participation in this study:

- presence of symptomatic brain metastases. Note: Subjects with symptomatic brain lesions who have been definitively treated with stereotactic radiation therapy, surgery or gamma knife therapy are eligible;
- presence of active brain metastases defined as new or progressive brain metastases at the time of study entry. Note: Subjects with treated or stable brain metastases are eligible;
- presence of leptomeningeal metastases;

- presence of malignant pleural effusion or ascites;
- systemic chronic steroid therapy (> 10mg/day prednisone or equivalent) or any other immunosuppressive therapy a within 7 days prior to leukapheresis or 72 hours prior to infusion of the MC2 TCR T cells. Note: Local steroids such as topical, inhaled, nasal and ophthalmic steroids are allowed;
- active, known or suspected autoimmune disease or a documented history of autoimmune disease. Note: Subjects with vitiligo, controlled type I diabetes mellitus on stable insulin dose, residual autoimmune-related hypothyroidism only requiring hormone replacement or psoriasis not requiring systemic treatment are permitted;
- any active systemic infections, coagulation disorders or other active major medical illnesses, such as active autoimmune diseases requiring anti-TNF treatment;
- history of myocardial infarction, cardiac angioplasty or stenting, unstable angina, or other clinically significant cardiac disease within 6 months of enrollment;
- AEs of previous treatment. Toxicities associated with prior systemic and non-systemic treatment must have recovered to a grade 1 or less. Patients may have undergone minor surgical procedures or palliative radiotherapy (for non-target lesions) within the past 4 weeks, as long as all toxicities have recovered to grade 1 or less;
- women who are pregnant or breastfeeding. A negative pregnancy test before inclusion in the trial is required for all women of child bearing age;
- use of any live vaccines against infectious diseases within the last 3 months;
- active infection requiring systemic antibiotic therapy at start of study treatment;
- prior allogenic bone marrow or solid organ transplant;
- history of known hypersensitivity to any of the investigational drugs used in this study;
- malignant disease, other than being treated in this study. Exceptions to this exclusion include the following: malignancies that were treated curatively and have not recurred within 2 years prior to start of study treatment; completely resected basal cell and squamous cell skin cancers and any completely resected carcinoma in situ.

4.4 Sample size calculation

In phase I, patients will be treated according to an accelerated titration phase-I trial [107] in subsequent cohorts of 1 patient. In case a DLT is encountered at a given dose level, the study is converted to a conventional 3+3 phase-I trial design. From that moment on, dose escalation will be semi-logarithmically to MTD (up-escalation, with DLT in 1 of the 6 patients, and 1 or 2 down-escalations with DLT in 2 of the 6 patients).
If in the 3+3 phase I trial design, a DLT is encountered in one of the first 3 patients at a given dose level, additional patients to a maximum of 6 are enrolled at that dose level. Escalation to the next higher dose level only occurs when the DLT rate is below 33%. The MTD is defined as the highest dose that can be safely administered yielding no DLT or a DLT rate below 33%.

In phase II, patients will be treated at the MTD or highest T cell dose evaluated according an optimal Simon-2 stage approach. Sample size calculation is based on the percentage of patients with an objective clinical response according the RECIST v1.1. The following statistical values will be adhered to: p0 = 0.05 (probability of spontaneous clinical response); p1 = 0.35 (expected probability of therapy-induced clinical response); alpha = 0.05 and power 0.8. The p0 value is set at 0.05 since we expect that without epigenetic pretreatment no spontaneous clinical responses will be observed.

In case no clinical responses are observed in the first 6 evaluable patients, the present study will be closed and the conclusion will be that in the applied setting T cell treatment lacks efficiency; at 1 or more clinical responders, patient numbers are extended to 12 (including the first stage). If the total number of responses for the two stages combined is less 3, the trial will be stopped as soon as this is evident and the conclusion will be that in the applied setting the T cell treatment lacks efficiency; at 3 or more responses in 12 evaluable patients the treatment is considered to show activity and warrants further investigations. Again safety data will be evaluated by the DMC at the end of the trial.
Patients who cannot be evaluated for response will be replaced by new patients.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Patients will first undergo leukapheresis for retrieval of T cells. Once transduction with the retroviral vector encoding the MC2 TCR has been successfully employed (meeting the in-process quality criterion, i.e., ≥10% surface expression of TCR transgenes on T cells), patients will be treated according to the protocol. This protocol consists of treatment with the epigenetic drugs AZA and VP to upregulate the expression of MC2 in tumor cells and to induce a mild state of lymphopenia, followed by infusion of autologous TCR T cells, as shown in Figure 2.

5.2 Use of co-intervention (if applicable)

Patients will be treated exactly according to protocol (see § 7).

5.3 Escape medication (if applicable)

Nevertheless, In case patients may experience a cytokine release syndrome with severe symptoms in the current study, patients will be treated with high dose corticosteroids, the anti-IL6R antibody tocilizumab[7], and the anti-CD52 antibody alemtuzumab, depending on the severity of the symptoms.

6. INVESTIGATIONAL PRODUCT

Patients will receive autologous MC2 TCR T cells, which will be produced according to the IMPD (see IMPD: MC2 TCR transduced T cells, version 2.0). Transduction and expansion of T cells will be performed at EMC, again according to IMPD. After the expansion phase, T cells are washed, concentrated and released by the QP gene therapy (pharmacist). Within 6 hours, MC2 TCR T cells will be intravenously administered to the patient, without further controls and/or processing, which has been previously validated (see IMPD).

For this IMP resides under environmental licences IM-MV 19-004 (LG 19-003) – application under consideration since February 21, 2019

Name and description of investigational product(s)

The Investigational product is : MC2 TCR T cells.

The MC2 TCR T cells are autologous T cells that have been transduced with the pMP71 retroviral vector expressing the codon optimized $MC2_{336\text{-}344}$ specific T cell receptor TCR16. The α and β chains of the TCR16 are expressed in equimolar amounts from the same vector using the picornavirus 2A cleavage element.

TCR16-positive CD4+ and CD8+ T cells can be visualized in flow cytometry (FCM) analysis, using the TCR16Vb-specific mAb (anti-TCRVb3). As TCR16 is CD8-dependent, FCM analysis using the TCR16-specific pMHC-multimer (HLA-A2/$MC2_{ALK}$), accurately enumerates TCR16+CD8+ T cells, but not TCR16+ CD4+ T cells.[54, 67, 68] The assessment of the T cell dose for patient treatment will be based on the FCM analysis of the TCRVb3+ (CD4+ and CD8+) T cells (corrected for the naturally occurring number of TCRVb3+ T cells in that particular patient)

Summary of findings from non-clinical studies

When introduced into primary human T cells, the MC2 TCR16αβ genes were functionally expressed on the cell membrane. Our in vitro studies revealed that the MC2 TCR16 displays a low-to-intermediate ligand-binding affinity (ED50=0.75 nM) and a dependency for the CD8α co-receptor. Furthermore, MC2 TCR16 T cells, when incubated with native MC2/A2+ tumor cells, displayed antigen-specific cytolysis and INFγ production. TCR expression and function could be maintained in cells kept in culture for longer than 3 weeks.

Pre-treatment with AZA+VP significantly increased MC2 gene expression in melanoma and HNSCC tumor cell lines, but not normal cells, and enhanced their ability to elicit an INFγ response by MC2 TCR T cells.

Evaluation of toxicity risks for the MC2 target antigen as well as its corresponding TCR16 revealed that:

- MC2 is not expressed on/in non-gonadal somatic healthy tissues;
- TCR16 has a highly stringent TCR recognition motif towards the cognate antigen

Collectively, these analyses point towards a beneficial safety profile for MC2 TCR16.

Summary of findings from clinical studies

This is the first in man application of MC2 TCR T cells, so no clinical data are available.

In previous clinical trials, TCRs were usually restricted to the HLA-A1 or A2 genotype and directed against differentiation antigens (i.e. MART-1, gp100, CEA, and p53), Cancer Germline Antigens (i.e. MAGE-A3 and NY-ESO-1), and viral antigens. Collectively, these trials demonstrated responses in 12 to 80% of patients with metastatic melanoma, colorectal carcinoma, synovial sarcoma, and multiple myeloma.[2-4] However, treatment-related AEs became evident from studies with TCRs, in particular for TCRs with high-affinity, directed against antigens with over-expression in both tumors and healthy tissue.[5, 50-52]. Two previous clinical studies applied TCRs directed against MAGE-A3 (2 different epitopes) and reported fatal neurological and cardiovascular AEs [5, 52]. These AEs were most likely caused by the use of affinity-enhanced TCRs that recognize a shared or highly similar epitope compared to the cognate epitope.

Summary of known and potential risks and benefits

For patients with unresectable stage IIIc or stage IV melanoma and patients with unresectable stage III Head and Neck Squamous Cell Cancer (HNSCC), who have failed standard therapies (including immune check point blockade), prognosis is generally poor. TCR gene therapy could provide an attractive alternative since it is a strategy that enables the fast and efficient generation of large numbers of tumor reactive T cells. In patients with melanoma, AT using autologous TCR T cells with affinity enhanced TCRs for MAGE-A3 has shown clinical responses, but also fatal on- and off target toxicities as a result of either targeting a common epitope of the MAGE-A antigen superfamily or a TCR with a non-restricted binding motif. In the current study, these (S)AEs are not expected as the MC2-specific TCR has a restricted binding motif without enhanced affinity. In this patient population, the potential burden and AEs are justified by the substantial chance of (durable) tumor responses as a result of the proposed treatment. See also §1.3.4.

Description and justification of route of administration and dosage

The in vitro generated and expanded MC2 TCR cell product is resuspended in a volume of 200 ml Ringers lactate w/ 1% HSA for direct intravenous administration. Administration of blood products, including (gene modified) T cell products, is by default intravenously. Administration of the fresh product optimally preserves (gene modified) T cell properties. The staring dose of in the phase I part is $5x10^7$ MC2 TCR T cells. This low dose is chosen as this is the first in man application of MC2 TCR T cells and SAEs have been reported at 100-fold higher doses of TCR T cells, albeit with other specificity. As MC2 has been selected after careful evaluation of safety aspects of the receptor TCR16, no AEs are expected at this dose.

Dosages, dosage modifications and method of administration

The phase I starts at an MC2 TCR T cell dose of $5x10^7$ and will be further escalated, based on toxicities and may be escalated up to $5x10^{10}$ MC2 TCR T cells. The MC2 TCR Tcells will be

administered in a single intravenous infusion at treatment day 0, with support of IL-2 administrations (s.c. $5x10^5$ IU/m^2 2qd) at treatment days 0 to 4.

Preparation and labelling of Investigational Medicinal Product

The MC2 TCR T cell product is produced at the Erasmus MC Facility for Cell- and Gene-Therapy. At the end of production the MC2 TCR T cells are harvest from culture and resuspended in 200 ml of infusion medium (Ringer Lactate containing 1% human serum albumin) in an infusion bag, for direct administration. Patient product specific labels will be adhered to the infusion bag and the 'outer bag', in which the infusion bag is transported (for product label, see appendix 2; for QP release form, see IPMD Appendix 6).

Drug accountability

The production of the MC2 TCR T-cell product is performed by qualified ATMP production personnel. At the end of production, the head of production will summarize the results of the production and QC tests on the release form. The head of QA/QC will verify whether the analyses have been performed correctly. The release of the MC2 TCR T cell product is performed by the QP for cell and gene therapy products. The release of the MC2 TCR T cell end-product is based on results of control tests from both intermediate cell products and the end-product. The MC2 TCR T cell end-product will only be released when pre-set release criteria are met (see IMPD Table 11).

Out of Specification (OOS)

In case the Investigational product (autologous MC2 TCR T cell) characteristics are out of specification (regarding cell number, viability or proportion MC2 TCR+ T cells), yet with a positive benefit/risk-analysis, the QP and treating physician may still decide to release the product for patient administration. Patients receiving an OOS investigational product will be included in the safety analysis, but excluded from efficacy analysis. An OOS investigational product administration will be notified to the competent authorities (see also SOP CK07.1150).

7. METHODS

7.1 Study endpoints

7.1.1 Phase I - primary study endpoints

- AEs according to CTCAE v5.0;
- recommended phase II dose;
- feasibility to deliver this sequence of treatment.

7.1.2 Phase II - primary study endpoint

- objective response rate according to RECIST v1.1 Response is defined as complete remission (CR) and partial response (PR) according to RECIST v1.1.

7.1.3 Phase II - secondary endpoints

- PFS, defined from the date of administration of TCR T cells to the date of first documented progressive disease according to RECIST v1.1 or death. Patients who are alive and progression-free at the time of the analysis will be censored at their last available assessment date;
- OS, defined from the date of administration of TCR T cells to the date of death. Patients who are alive at the time of the analysis will be censored at the date they were last known to be alive.

7.1.4 Phase I and phase II - Secondary and exploratory endpoints

- persistence of MC2 TCR T cells, their differentiation state and ability to recognize tumor cells at regular intervals (see § 7.3.3) following start of T cell treatment;
- presence of immune and T cell parameters in blood, and micro-environment of tumor tissues using advanced flow cytometry and in situ stainings;
- MC2 expression in tumor tissues (when available) prior to and after epigenetic drug treatment;
- global DNA hypomethylation and histone acetylation in PBMC after epigenetic drug treatment;
- response rate according to immune related Response Criteria (irRC).[106]

7.2 Randomisation, blinding and treatment allocation

Patients fulfilling eligibility criteria will be enrolled in the study. Patients have to be registered before leukapheresis is performed.

7.3 Study procedures

A brief overview of the study procedures is presented in Figures 2 and 3.

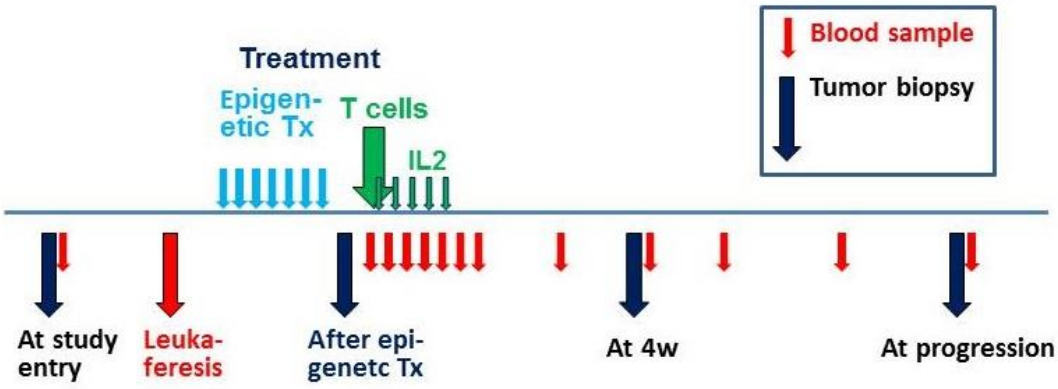

Figure 3. Treatment and monitoring scheme, for more detail see Tables 1a/b

7.3.1 Pre-study screening

Screening will comprise two steps, first subjects will be screened for HLA-A2 status; and if positive, subsequently for MC2 expression in the tumor. The screening period begins once written informed consent is provided. An attempt will be made to complete all procedures within 30 days of enrollment. However, given the multiple screening tests and the pre-study procedures to assess patient eligibility, tests older than 30 days may be acceptable to limit unnecessary test repetition. If older than 30 days,

at least laboratory tests and CT scans need to be repeated. Other key tests can be repeated at the discretion of the treating physicians.

The following screening tests will be performed.
Step 1:

- HLA typing;
- in case no archival tumor material is available a fresh biopsy of a melanoma or HNSCC lesion accessible to sampling will be collected;
- tumor evaluation by immunohistochemistry for MC2 protein expression.

Step 2: in case patient is HLA-A2 positive and has >1% MC2 expression in tumor tissue, the remaining baseline parameters as defined in § 4.2 and 4.3 are evaluated and include:

- complete physical examination including height, weight, performance status, and vital signs;
- laboratory evaluations:
  - hematology: hemoglobin, Ht, erythrocytes, reticulocytes, platelets, WBC and differential;
  - blood chemistry: Na, K, creatinine, Ca, P, Mg, bilirubin, AF, GT, ASAT, ALAT, LDH, glucose, APTT, INR, fibrinogen;
  - urine analysis (pH, protein, erythrocytes, leucocytes, glucose, ketones, nitrites)
  - TSH and FT4;
  - b-HCG pregnancy test for all women of child-bearing age;
- baseline CT of the chest, abdomen pelvis and brain MRI. Additional imaging may be performed if clinically indicated based on patient's signs and symptoms;
- ECG;
- HIV, HCV, CMV, HAS antibody titers, HbsAG, EBV panel and lues panel;
- in case no fresh biopsy has been collected for screening, a baseline excision or biopsy of a melanoma or HNSCC lesion accessible to sampling will be collected.

7.3.2 Procedures of TCR T cell treatment

Pre-treatment phase - leukapheresis and T cell transduction

Prior to study treatment, at day -13, patients will undergo a 10 liter leukapheresis to collect a minimum of $1x10^9$ mononuclear cells (MNCs) and 300 ml plasma. In addition, 70 ml blood in serum tubes will be collected. MNCs are isolated from the leukapheresis product by Ficoll gradient separation; at least $6x10^8$ of MNCs are freshly processed to prepare the TCR T cells, the remainder is cryopreserved and serves as back-up. The plasma and the serum are both used as autologous cell culture medium additive during the preparation of the TCR T cells. Patient MNCs will be transduced and processed in accordance with the cell production facility's SOPs as described in the IMPD (days -14 to day 0), and have to be released for clinical application by a Qualified Person gene therapy. In process and final product samples are cryopreserved for retrospective testing and archive.

Treatment schedule

Treatment schedule will be according the following scheme, which will comprise a course of therapy from day -13 through day 0 (also see Tables 1a/b).

Day -9 to day -3

5-azacitidin (AZA): 75 mg/m^2/day subcutaneously for 7 days.

Valproic acid (VP): 50mg/kg/day per os for 7 days.

Granisetron: 1 mg/day per os (supportive care medication during AZA and VP treatment).

Note: patients with R/M HNSCC that are unable to swallow, will receive Valproic acid in feeding by stomach tube and granisteron 10-40 µg/kg. i.v.

Day 0

TCR T cells: depending on dose level (phase I) and MTD (phase II), a single dose of between 5x10^7 and 5x10^{10} T cells will be administered intravenously over 20 to 30 minutes. Cell preparation is detailed in the IMPD.

Day 0 to day 4

Interleuki-2 (IL-2): 5x10^5 IU/m^2/evry 12 hours subcutaneously for 5 days.

7.3.2 On study evaluation

During the preparative epigenetic regimen: every 2 days

- physical examination, laboratory evaluations (Hematology and Chemistry) and assessment of AEs, see § 7.3.1.

Prior to T cell infusion: day 0

- physical examination, laboratory evaluations (Hematology and Chemistry) and assessment of AEs, see § 7.3.1.

After T cell Infusion

- vital signs will be monitored hourly until stable and then routinely every 4 hours up to 24 hours. Thereafter, every day until hospital discharge, unless otherwise clinically indicated;
- laboratory evaluations (Hematology and Chemistry), see § 7.3.1; daily until hospital discharge;
- blood samples (4ml) are obtained to study systemic release of inflammatory cytokines and presence of MC2 TCR T cells, i.e., daily until hospital discharge. Thereafter, these assays will be performed on blood samples obtained as described in § 7.3.3.

7.3.3 Additional immune-related research evaluations

At regular intervals prior to and after T cell infusion 40ml blood samples will be obtained for immune-related exploratory testing and archiving, i.e., at day -13, day 0 (prior to infusion of TCR T cells), and post T cell infusion at days 7, 14, 21, 28, 60 and 90 subsequently every 3 months until 1 year, and thereafter yearly until 5 year after T cell infusion. From blood PBMC and plasma are isolated and cryopreserved. Blood samples will be analyzed as follows:

Blood and PBMC

- freshly collected blood samples will be subjected to multiplex flowcytometry to assess:
  - numbers of 18 leukocyte subsets, and
  - Presence of MC2 TCR T cells by $pMHC_{HLA\text{-}A2/ALK}$ binding and qPCR;
- cryopreserved PBMC will be subjected to multiplex flowcytometry for in-depth T cell immune profiling, including, amongst others, assessment of differentiation, expression of inhibitory as well as stimulatory co-signaling molecules;
- immune profile, as above, of MC2 TCR T cells;
- cryopreserved PBMC will be tested for MC2 TCR mediated immune-functions, e.g., interferon-γ production upon encounter of MC2-expressing tumor cells, and
- TCR repertoire (by RNAseq).

Plasma

- plasma samples will be tested with ELISA or multiplex assays for cytokines (such as IFNγ, IL-2, TNFα, IL-6, IL-10, CXCL10 or CXCL9). IL-6 will be measured every day during hospitalization and will continue up to 1 month after T cell infusion. At that time, the IL-6 levels are normalized again in all previous patients.

Tumor biopsy

An 18G needle biopsy or a thru-cut biopsy will be taken from a metastasis before and after treatment (at 3 weeks after T cell infusion and at progression; optional following epigenetic treatment), when feasible from the same lesion. Tissue will be processed for future NGS analysis using in silico tools to assess mutational burden, TCR repertoire and expression of immune-related genes. In addition, tissue will be processed for multiplex in situ stainings using the Vectra technology to assess the immune micro-environment of tumor tissue (such as presence of immune effector cells, among which TCR T cells; immune suppressor cells; immune checkpoints; and MC2 antigen).
Tumor biopsies are per protocol, but may be derogated from in consultation with the PI.

7.3.4 Post-treatment and off-study evaluation

After re-infusion of T cells, response evaluation will be performed at 6 weeks (± 1 week), 12 weeks (± 1 week), and then every 3 months (± 1 weeks). Patients with progressive disease will be monitored at the same time points until death or next systemic treatment.

At each follow-up moment, the following evaluations will be performed:

- physical examination including vital signs and ECOG status;
- toxicity assessment;
- CT of the chest, abdomen and pelvis; this evaluation will be used to determine tumor response. If clinically indicated, other scans may be performed, e.g. MRI of the head and neck region and/or brain;
- laboratory evaluations: hematology and chemistry, see § 7.3.1;
- other tests will be performed as clinically indicated.

7.3.5 Treatment of treatment-related toxicity

7.3.5.1 Treatment of TCR T cell-related toxicity

Although this study is designed to limit chances of on- or off-target toxicities, we cannot formally exclude the development of T cell-related AEs, which usually resemble autoimmune disorders. In addition, and again against expectations, patients may develop signs of CRS with high fevers, chills/rigors and hypotension. In case these patients are not responding to supportive measures, patients will be treated with high doses of corticosteroids (prednisone 1-2 mg/kg). In case of high serum levels of IL-6 (> 200 pg/ml), patients will be treated with tocilizumab (8 mg/kg with maximum of 800 mg). In case of an acute deterioration of the patient's condition, not due to septic shock syndrome, patients will additionally be treated with alemtuzumab (3 mg day 1, 10 mg day 2, 30 mg day 3 and later), until T cell count drops to <50 CD3 cells/microliter) and the patient's situation is stabilized.

7.3.5.2 Treatment of epigenetic durg-related toxicity

Patients will receive anti-emetic medication. Depending on the nature of the observed toxicity appropriate additional supportive measures will be taken.

7.3.5.2 Treatment of IL-2-related toxicity

Depending on the nature of the observed toxicity appropriate additional supportive measures will be taken.

7.3.6 Re-treatment

A single re-treatment (including pretreatment with epigenetic drugs) is permitted for patients with relapse after an initial complete or partial response after treatment with TCR T cells . Period between first treatment cycle and re-treatment should be at least 6 months. Patients must meet all original eligibility criteria (including hematological recovery) and have additional T cells available. The eligibility criteria can be found in § 4.2 and 4.3.

7.3.7 Off-study criteria

Patients will be taken off-study when one of the following criteria is met:

- patient voluntarily withdraws;
- deterioration of performance status not being able to undergo (pre-)treatment;
- the final product of TCR T cells does not meet all release criteria;
- significant patient non-compliance;
- progressive disease without eligibility for re-treatment;
- if the treating physician is convinced that it is in the best interest of the patient to discontinue study participation.

7.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator or treating physician can decide to withdraw a subject from the study for urgent medical reasons.

7.4.2 Specific criteria for withdrawal

If, after having provided informed consent but before initiation of the treatment, patients do not fulfil the eligibility criteria anymore, patients will be withdrawn from the study.

7.4.3 Replacement of individual subjects after withdrawal

Patients who are withdrawn prior to initiation of the treatment may be replaced.

7.4.3 Follow-up of subjects withdrawn from treatment

Follow-up of patients withdrawn from the study will continue at least until 6 weeks after completion of treatment or until treatment-related toxicity has resolved to grade 2 or less. Thereafter, patients will receive standard follow-up.

7.5 Premature termination of the study

- If 2 patients develop a non-manageable grade 4 toxicity due to the treatment, which does not show improvement within 48 hours with supportive measures, or are clearly not known as side effects of the preparative regimen or not related to events external to participation in this study, accrual to the study will be halted pending discussions with the DMC.
- In case of a grade 5 toxicity not attributable to disease progression or more than one prolonged grade 4 major organ toxicity (lasting more than a month after treatment) prior to completion of the study will halt patient treatments pending a review of those toxicities by CCMO and DMC.

7.6 Long-term follow-up

Per protocol follow-up is 5 years after T cell infusions.

Table 1a. Schedule of assessments

Parameters Day	Baseline Screening -28	Leuka-feresis -13	Epigenetic therapy -9 to -2	T cell infusion 0	Day 1 to 7[1]	Follow-up[2]
Informed consent	X					
HLA typing	X					
Pathological confirmation of melanoma or HNSCC and MAGE-C2 expression in primary tumor and/or metastasis (archival or fresh biopsy)	X					
Physical examination[3]	X	X[4]	X[5]	X[6]	X[6]	X
Hematology: ANC, Hb, platelet count	X	X	X	X	X	X
Chemistry[7]	X	X	X	X	X	X
Thyroid: TSH and FT4		X				
Coagulation: PT/INR and aPTT		X				
b-HCG pregnancy test, if applicable[8]	X	X				
Urinalysis and culture[9]		X				
Serology[10]	X					
CT chest, abdomen, pelvis and evaluation of skin lesions	X					X
Brain MRI[11]	X					X
ECG	X					
Adverse events			X	X	X	X
Biopsy of metastasis[12]		X		(X)		X
Blood for immunological testing[13]		X		X	X	X

1 Every day until release from hospital, expectedly up to day 7
2 At days 7, 14, 21, 28, 60 and 90 subsequently every 3 months until 1 year, and thereafter yearly until 5 year after T cell infusion.
3 WHO, PS, weight, temperature, pulse, blood pressure
4 May be repeated at the discretion of investigator if screening exams are older than 30 days
5 At time of epigenetic treatment: ECOG should be 0 to 1
6 After cell infusion: vital signs to be monitored hourly until stable, then routinely (every 4 hours) unless otherwise clinically indicated
7 Na, K, Cl, Ca, Mg, HCO3-, Phosphate, Alkaline Phosphatase, Direct bilirubin, LD, Total Protein, CK, Uric acid. At screening only ALAT, ASAT, serum creatinine, total bilirubin, CRP.
8 Within 1 week of epigenetic treatment
9 If clinically indicated
10 HIV, HbsAG, HCV, CMV, HSV, EBV and lues
11 Needs to be repeated for melanoma patients if older than 30 days at start of study treatment. In that case, CT is allowed instead of MRI. Of note: at other time points, CT is not sufficient for brain imaging and MRI brain is required.
12 Biopsy of accessible tumor site (18G or thru-cut) to be taken for translational research before and after treatment (at 3 weeks after AT and at progression), when possible of same site; Optional: an additional biopsy may be taken at day 0, i.e, after epigenetic treatment and before MC2TCR T cell infusion. Tumor biopsies are per protocol, but may be derogated from in consultation with the PI.
13 Samples with a maximum of 40ml EDTA blood

Table 1b Treatment phase schedule and event monitoring calendar Days -14 to +9

Parameters / Day	Day of Treatment																		
	-14	-9	-8	-7	-6	-5	-4	-3	-1	0	1	2	3	4	5	6	7	8	9
Inpatient care										X	X	X	X	X	According to clinical need				
Outpatient care	X	X	X	X	X	X	X	X											
Epigenetic drugs		A/V	A/V	A/V	A/V	A/V	A/V	A/V											
MC2 TCR T cells										X									
Interleukin-2										X	X	X	X	X					
Physical examination Adverse events	X	X		X		X		X		X	X	X	X	X	According to clinical need				
Hematology	X	X		X		X		X		X	X	X	X	X	According to clinical need				
Chemistry	X	X		X		X		X		X	X	X	X		According to clinical need				
Coagulation screen		X								X	According to clinical need								
Leukapheresis[14]	X												According to clinical need						
Blood (serum)[15]	X																		
Blood for cytokine assays T cell persistence[16]										X	X	X	X	X	Untill discharge				
Blood for immunological testing and archive[13]		X						X		X							X		

Blood collections:
13 Samples with a maximum of 40ml EDTA blood
14 PBMC harvest including 300 ml plasma from 10 liter leukapheresis
15 70ml blood in serum tubes, for MC2 TCR T cell generation
16 Samples with a maximum of 3 ml EDTA blood

8. SAFETY REPORTING

8.1 Temporary halt for reasons of subject safety

In accordance to § 10, subsection 4, of the Medical Research Involving Human Subjects Act (WMO), the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject's health or safety. The PI will notify the CCMO without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the CCMO. The investigator will take care that all subjects are kept informed.

8.2 Adverse and serious adverse events

An investigator who is a qualified physician will evaluate all AEs according to the NCI Common Terminology for Adverse Events (CTCAE), version 5.0. The CTCAE grade at onset and the maximum CTCAE grade of any AE will be recorded on AE case report forms/worksheets.

8.2.1 Adverse events (AEs)

AEs are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product/trial procedure/the experimental intervention. All AEs reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

8.2.2 Serious adverse events (SAEs)

A SAE is any untoward medical occurrence or effect that:

- results in death;
- is life threatening (at the time of the event);
- requires hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

Hospitalization is part of the treatment procedure, starting with the administration of the T cell product, and will therefore not be reported as SAE. If the patient experiences an event that falls under one of the above-mentioned criteria and/or the event requires IV or other treatment that cannot be given outside the hospital and/or the patient is admitted to an Intensive Care Unit, the event will be reported as an SAE. An elective hospital admission will not be considered as SAE.

Note: Suspected unexpected serious adverse reactions (SUSARs) and SAEs are not only those linked with the IMP itself (MC2 TCR T cells), but also those in relation to the following aspects: quality defects of the IMP leading or potentially leading to serious events, serious events occurring during the

donation (leukapheresis), treatments carried out in view of the treatment with the IMP (epigenetic drug treatment), and any other procedures which do not take place outside of the clinical trial.

8.2.3 Reporting of SAEs

The investigator will report the SAEs through the web portal *ToetsingOnline* to the CCMO that approved the protocol (CCMO) within 7 days of first knowledge of the SAEs that result in death or are life threatening, which is followed by a period of maximally 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the SAE.

8.3 Suspected unexpected serious adverse reactions (SUSARs)

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 8.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the IMP, regardless of the administered dose;
    - the adverse reaction must be unexpected, that is to say the nature and severity of the adverse reaction are not in agreement with the product information (see IMPD).

8.3.1 Reporting of SUSARs

The sponsor will provide expedited reporting of SUSARs and any follow-up information electronically through the above-mentioned web portal ToetsingOnline.
SUSARs that are fatal or life-threatening will be reported to the authorities within 7 days for a preliminary report, with another 8 days for completion of the report. All other SUSARs will be reported not later than 15 days after the investigator has first knowledge of the SUSAR.
Additional information can be sent as soon as possible.

8.4 Annual safety report

The PI will submit, once a year throughout the clinical trial, a safety report to the CCMO and competent authority.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the IMP under investigation.

8.5 Follow-up of AEs

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow-up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study.

8.6 Data Monitoring Committee (DMC)

A DMC will be installed and will be comprised of at least 3 (inter)national experts, including at least 2 medical oncologists, in the field of adoptive T cell therapy, and will be installed once the protocol has been approved by CCMO. Members of the DMC are Prof. Carl June, Parker Institute for Cancer Immunotherapy, University of Pennsylvania, Philadelphia, PA, USA; Fiona Thistlethwaite, The Christie NHS Foundation Trust, Manchester, UK and Prof John Haanen, NKI, Amsterdam.

The DMC will evaluate the safety data after completion of phase I part, after the first stage in the phase II part (6 patients) and at the end of the second stage of the phase II part (12 patients) according § 9.1 and DMC charter version 1.0 dated 3 june 2019. DMC may also be consulted at the discretion of the treating physicians.

The DMC will meet in TCs and will decide on the recommended dose for the phase II part and whether further patient inclusion for phase II, second stage is warranted. Rules for stage evaluation and pre-termination of the study due to AEs can be found in § 8.3 of the protocol.

The advice(s) of the DMC will only be sent to study project team. If the project team decides not to fully implement the advice of the DMC, the project team will send the advice to the CCMO, including a note to substantiate why (part of) the advice of the DMC will not be followed.

9. STATISTICAL ANALYSIS

All analyses will be done in accordance with the per protocol principle, restricted to eligible and evaluable patients.

9.1 Primary study parameter(s)

With respect to the main endpoint: A binomial probability test will be used to evaluate whether this regimen is worth further study. A 90% confidence interval for the percentage of response will be presented ($\alpha = 0.10$).

In phase I, patients will be treated according to an accelerated titration phase-I trial [107] in subsequent cohorts of 1 patient. In case a DLT is encountered at a given dose level, the study is converted to a conventional 3+3 phase-I trial design. If in the 3+3 phase I trial design, a DLT is

encountered in one of the first 3 patients at a given dose level, additional patients to a maximum of 6 are enrolled at that dose level. Escalation to the next higher dose level only occurs when the DLT rate is below 33%. The MTD is defined as the highest dose that can be safely administered yielding no DLT or a DLT rate below 33%.

In phase II, patients will be treated at the MTD or highest T cell dose and evaluated according an optimal Simon-2 stage approach. Sample size calculation is based on the percentage of patients with an objective clinical response according to RECIST v1.1. The following statistical values will be adhered to: p0=0.05 (probability of spontaneous clinical response); p1=0.35 (expected probability of therapy-induced clinical response); alpha =0.05 and power 0.8. The p0 value is set at 0.05 since we expect that without epigenetic pretreatment no spontaneous non-T cell related clinical responses will be observed.

In case no clinical responses are observed in the first 6 evaluable patients, the present study will be closed and the conclusion will be that in the applied setting T cell treatment lacks efficiency; at 1 or more clinical responders, patient numbers are extended to 12 (including the first stage). If the total number of responses for the two stages combined is less than 3, the trial will be stopped as soon as this is evident and the conclusion will be that in the applied setting T cell treatment lacks efficiency; at 3 or more responses in 12 evaluable patients the treatment is considered to show activity and warrants further investigations.

9.2 Secondary study parameter(s)

PFS, OS and duration of response will be explored by means of the Kaplan-Meier method. For the exploratory study parameters, descriptive statistics will be applied.

9.3 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of AEs, see § 7.5.

9.4 Interim analysis (if applicable)

Not applicable.

10. ETHICAL CONSIDERATIONS

10.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki, (Declaration of Helsinki, 59th WMA General Assembly, Seoul, October 2008) and in accordance with the Medical Research Involving Human Subjects Act (WMO).The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice

(ref:http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf).
The protocol must be approved by the CCMO and competent authority (CA) prior to starting this study.

10.2 Recruitment and consent

Recruitment
It is the responsibility of the investigator to give each patient, prior to entering the trial, full and adequate verbal and written information (see below). Confidentiality-related information will be provided. The written information must be given to each patient. Patients will be given sufficient time to consider the provided text. An independent physician will be available for consultation. It is the responsibility of the investigator to obtain signed informed consent from every patient prior to the start of any study-related procedure(s).

Informed consent (IC)
All patients will be informed about the aims of the study, the possible adverse events, the procedures and possible hazards to which he/she will be exposed, and the mechanism of treatment allocation (patient information letter). They will be informed as to the strict confidentiality of their patient data, but that their medical records may be reviewed for trial purposes by authorized individuals other than their treating physician. It will be emphasized that the participation is voluntary and that the patient is allowed to refuse further participation in the protocol whenever he/she decides to do so. This will not prejudice the patient's subsequent care. Documented informed consent will be obtained for all patients included in the study before they are registered as having entered the study.
The IC procedure will conform to the ICH guidelines on Good Clinical Practice. This implies that "the written IC form will be signed and personally dated by the patient or by the patient's legally acceptable representative".

The written patient information is part of the documentation reviewed by the CCMO. The patient information letter and informed consent form are attached as a separate document

10.3 Objection by minors or incapacitated subjects

Not Applicable.

10.4 Benefits and risks assessment, group relatedness

The prognosis of patients with unresectable stage IIIc or metastatic stage IV cutaneous and mucosal melanoma has improved in the past 5 years as result of of targeted therapy and immune checkpoint inhibitors. With these developments the poor prognosis has improved considerably. Still part of the patients, do not benefit from these novel therapies. In addition, treatment options for patients with metastatic uveal melanoma are still very limited.

Although treatment options for patients with R/M HNSCC have increased, the median overall survival still is less than 1 year. Besides, treatment of R/M HNSCC often is accompanied by considerable toxicity.

In patients with metastatic melanoma, synovial carcinoma and multiple myeloma, adoptive transfer of TCR T cells has shown limited AEs and significant objective clinical responses of 55, 61 and 80%, respectively[1-3]. In the present protocol, we target MC2 using TCR16 T cells in patients with advanced melanoma or HNSCC that express MC2. To sensitize tumor cells to T cell treatment, patients will be pretreated with the epigenetic drugs AZA and VP. T cell treatment will be supported with low-dose IL-2 administrations.

Epigenetic treatment-related toxicity has been demonstrated to be well manageable. In fact, the doses of the administered epigenetic drugs are precedented and well tolerated and have been reported to induce only a mild transient leucopenia and neutropenic fever.[8]
In case vigorous *in vivo* activation of infused T cells occurs, patients may (although given the design of the study not expected) experience some auto-immunity and/or CRS. Auto-immune reactions are treated by standard of care. Depending on the severity of CRS (fever, chills, hypotension) patients may be treated with high dose corticosteroids whether or not in conjunction with anti-CD52 antibody alemtuzumab. In case of severe deterioration of the patient's condition,escape medication consisting of anti-IL6R antibody tocilizumab.[7] may be given.
Low-dose IL-2 –related toxicities are limited and well managible. [96] In fact, the doses of the administered IL-2 are precedented and well tolerated and have been reported to induce only a transient thrombocytopenia and mild malaise (flu-like symptoms).
The fact that these patients may have a substantial chance of durable objective responses, which otherwise would not occur, justifies for the burden and possible toxicities.

10.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO. This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

10.6 Incentives

Not applicable.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

11.1 Handling and storage of data and documents

Subject identification and registration

Prior to enrollment in the study and prior to any study related procedure, the patient must personally sign and date the informed consent form. Each patient will be given a unique sequential study subject number not based on the patient's initials or birthdate. The key to the patient study number is safeguarded by the local investigator.

Patient registration will done at the Erasmus MC Clinical Trial Center and will only be accepted from authorised investigators or through their authorised data managers or authorised staff members. A patient can be registered only after verification of eligibility. Patients have to be registered before leukapheresis is performed. During the registration procedure eligibility criteria and items of patients' informed consent are checked.

The handling of personal data complies with the EU General Data Protection Regulation and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet Algemene verordening gegevensbescherming (AVG). Collaboration with a third party outside the EU requires a privacy assurance agreement complying with these regulations.

The Informed Consent Form will include a statement by which the patient allows the Sponsor's duly authorized personnel, the ethics review committee (IRB/ERC) or similar or expert committee, and the regulatory authorities to have direct access to original medical records which support the data on the CRFs (e.g. patient's medical file). This personnel, bound by professional secrecy, must maintain the confidentiality of all personal identity or personal medical information, according to confidentiality and personal data protection rules and in compliance with all applicable privacy laws, rules, and regulations.

Storage of patient material

Patient biomaterials will be coded (anonymized) and will be stored for a up to 30 years to be used current translational research or for future cancer research by the Erasmus MC (EMC) or the EMC in collaboration with third parties within The Netherlands, within the EU or outside the EU. After that period, materials may be destroyed as per responsibility of the PI.

Data management

Data will be collected using an eCRF (electronic case report form) designed for this study. According

to ICH guidelines for Good Clinical Practice (GCP), the trial monitor will check the specific CRF entries against the source documents, as specified in the study specific monitoring plan.

11.2 Monitoring and Quality Assurance

The study will be conducted and data are generated, documented, and reported in compliance with the accepted standards of GCP. Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified personnel and review of protocol procedures before the study. Data collected on the eCRF will be verified for accuracy by the local trial monitor according to the Monitor Plan. All data management procedures will be documented in detail in a study-specific Data Management Plan.

11.3 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the CCMO application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the CCMO and to the competent authority.
Non-substantial amendments will not be notified to the CCMO and the competent authority, but will be recorded and filed by the investigator.

11.4 Annual progress report

The investigator will submit a summary of the progress of the trial to the CCMO once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.

11.5 Temporary halt and (prematurely) end of study report

The investigator will notify the CCMO of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.
The sponsor will notify the CCMO immediately of a temporary halt of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the CCMO within 15 days, including the reasons for the premature termination.
Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the CCMO.

11.6 Public disclosure and publication policy

Prior to initiation, the study will be submitted to the Dutch National Trial register, which is a recognized register that is acknowledged by the WHO and International Committee of Medical Journal Editors (ICMJE) and to the NCI's PDQ® Cancer Clinical Trials Registry.
All the results will officially be published. Study investigators will be informed in writing prior to any written communication or oral presentation about the study and invited to give comments.

12. STRUCTURED RISK ANALYSIS

12.1 Potential issues of concern

a. Level of knowledge about mechanism of action

The mechanism of action is immunologic. The treatment applies autologous T cells that are genetically modified *ex vivo* to express a patient derived MC2-specific TCR. Upon reinfusion into the patient the MC2 TCR T cells will recognize and kill MC2-expressing tumor cells.
The MC2 TCR recognizes the immunogenic HLA-A2 restricted $MC2_{ALK}$ epitope. MC2 is not expressed in healthy tissues (with the exception of gonads, but these sites are immune-privileged and have no MHC expression). The expression of MC2 on tumor cells *in vivo* is upregulated by a preparative regimen of epigenetic drugs prior to the MC2 TCR T cell infusion. MC2 TCR T cell infusions will be followed by low-dose IL-2 administrations to support proliferation, function and survial of T cells in vivo.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

This is the first in man application of MC2 TCR T cells. MC2 has not be targeted before with adoptive T cell therapy (AT). Clinical TCRs tested so far in AT were mostly HLA-A1 or A2-restricted and directed against differentiation antigens (i.e. MART-1, gp100, CEA and p53), CGAs (i.e. MAGE-A3 or NY-ESO-1) and viral antigens. Collectively, these trials demonstrated tumor responses in patients with metastatic melanoma, colorectal carcinoma, synovial sarcoma and multiple myeloma that ranged from 12 to 80%.[3, 4] However, treatment-related toxicity became evident from studies with TCRs, in particular for TCRs with high-affinity, directed against antigens that were *over-expressed* on tumors but were also expressed (in some cases a highly similar antigen) on healthy cells.[5, 50-52]

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

The primary mechanism of MC2 TCR T cells has been illustrated by *in vitro* recognition and the killing of MC2 positive, but not MC2-negative human tumor cell lines.[54, 67, 68] In patients, direct tumor cell killing by MC2 TCR T cells will be assessed directly in tumor biopsies and indirectly by assessing pro-inflammatory cytokines in blood.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

The CGA MC2 has a restricted expression profile and is only expressed by various tumors cells, but not in healthy tissues, with the exception of gonads, which are immune-privileged having no MHC expression.

The MC2-specific TCR16, recognizing the ALK epitope in the context of HLA-A2, has a very restricted recognition motif towards the ALK epitope, and TCR cross-reactivity was only found towards MAGE-C1, a related CGA that also shows no expression in healthy non-gonadal tissues.[54]

e. Analysis of potential effect

In the phase I-part of the study, DLT and the recommended phase II dose (at MTD) will be assessed. The optimal dose of a particular T cell product may vary per type of T cell preparation (TIL, CAR T cells, and TCR T cells), patient preparative treatment and expression of the target antigen. As MC2 is not expressed in normal somatic tissues, no 'on-target' toxicities are foreseen (see also "c"). Therefore, we anticipate that the MTD / recommended phase II dose will be in the higher dose range.

f. Pharmacokinetic considerations

The duration of anti-tumor responses is related to the level of persistence of TCR T cells in the blood .[56, 57] Therefore, we have incorporated in the protocol strategies to enhance TCR T cell persistence.[68] There are no special issues regarding elimination of MC2 TCR T cells.

g. Study population

Adult patients with unresectable stage IIIc and IV melanoma (including unknown primary, mucosal and uveal melanoma), and unresectable recurrent/metastatic (R/M) HNSCC for whom no standard treatment is available (anymore).

h. Interaction with other products

Patients will receive an epigenetic drug preparative regimen consisting of AZA (75 mg/m^2/day s.c. x 7 days), and VP (50 mg/kg/day p.o. x 7 days). Two days following this regimen, patients will receive one single intravenous adoptive transfer of autologous MC2 TCR T cells. The epigenetic drug regimen will enhance MC2-protein expression in tumor cells, but will not affect MC2 TCR T cell activity. Following TCR T cell transfer, no systemic immune suppressive agents (> 10mg/day prednisone or equivalent) are allowed. Low-dose IL-2 administrations following MC2 TCR T cell infusions will support proliferation, function and survival of T cells in vivo.

i. Predictability of effect

In patients, tumor cell killing by MC2 TCR T cells will be assessed directly in tumor tissue and indirectly by assessing pro-inflammatory cytokines in blood. In addition, persistence of MC2 TCR T cells in blood will be assessed (mirrors clinical response in similar studies); as well as immune and T cell parameters in blood, and micro-environment of tumor tissues. To document the effect of the epigenetic drug preparative regimen (i) the MC2 expression in tumor biopsies taken prior to and after

epigenetic drug treatment and (ii) the global DNA hypomethylation and histone acetylation in PBMCs will be assessed.

j. Can effects be managed?

In case patients may experience CRS with severe symptoms, patients will be treated with high dose corticosteroids, the anti-IL6R antibody tocilizumab [7], and the anti-CD52 antibody alemtuzumab, depending on the severity of the symptoms. Toxicities of epigenetic drugs and IL-2 are expected to be mild and managible by standard supportive care.

12.2 Synthesis

In patients with advanced melanoma and other tumor types, prior clinical studies have shown efficacy of T lymphocytes directed towards tumor antigens. To this end, tumor reactivity can be imposed by the transfer of TCR genes into previously non-reactive T cells. The development of TCR T cell therapy critically depends on selection and validation of effective and safe T cell target antigens.
We selected the CGA MC2, that is highly expressed in melanoma and HNSCC, but not in normal mature tissues. MC2 can evoke clinically effective T cell responses, as demonstrated in previous vaccination studies. We confirmed MC2 expression in a series of tumor cells and its absence in somatic healthy tissues. In addition, we have shown that MC2 expression in tumor cells, but not normal somatic cells, can be epigenetically upregulated.

Next, we isolated the MC2-specific TCR16 from a melanoma patient who showed a complete remission following a MAGE-A1 and MAGE-A3 vaccination regimen, without any clinical toxicity. Upon transfer of TCR16 to primary human T cells, these T cells express TCR16 and are able to recognize and kill MC2-expressing tumor cells. CR16 has a highly restricted recognition motif toward its cognate peptide and cross-reactivity is only limited to MAGE-C1, i.e., a related CGA without expression in healthy non-gonadal tissues. Collectively, these analyses showed absent MC2 expression in somatic healthy tissues and a beneficial safety profile for MC2 TCR16 [54], therefore the development of on- or off-target toxicities in patients treated with autologous MC2 TCR16 T cells is highly unlikely.

In this phase I/II study, we will investigate the safety and feasibility of AT with autologous MC2 TCR T cells, combined with epigenetic drug treatment, in patients with MC2-positive advanced melanoma or HNSCC. The epigenetic preparative regimen is well established and induces mild side effects. Based on the tumor-restricted expression of MC2 and the beneficial safety profile for MC2 TCR16, the development of on- or off-target toxicities in patients treated with autologous MC2 TCR16 T cells is highly unlikely. Nevertheless, in case patients may experience CRS with severe symptoms, patients will be treated with high dose corticosteroids, the anti-IL6R antibody tocilizumab, and the anti-CD52 antibody alemtuzumab, depending on the severity of the symptoms. Toxicities of epigenetic drugs and IL-2 are expected to be mild and managible by standard supportive care.

In this patient population, the potential burden and AEs are well justified by the substantial chance of (durable) tumor responses as a result of the proposed treatment.

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14. APPENDICES

Appendix 1: Eastern Cooperative Oncology Group (ECOG) Performance Status

Grade Description

0 Fully active, able to carry on all pre-disease performance without restriction.

1 Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).

2 Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.

3 Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.

4 Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.

5 Death

To be eligible for inclusion, patients have to have an ECOG performance status of 0 to 1.The ECOG performance status will be recorded throughout the study.

Appendix 2: Investigational product label

INVESTIGATIONAL PRODUCT
MAGE-C2 TCR16 getransduceerde autologe T cellen
Studie: NL69911.000.19 Studienummer: XXX

Patiëntnaam	:	……..
PID nummer patiënt	:	……..
Product	:	**MC2 TCR16 T cellen**
Aantal getransduceerde T cellen:		……. x 10^8
Volume product	:	……. ml*
Datum uitgifte	:	….. / …. / ……..
Houdbaar tot	:	… uur

Bewaartemperatuur product tussen 2-8ºC
Houdbaarheid product: maximaal 6 uur tussen 2 – 8ºC, waarvan maximaal 1 uur op kamertemperatuur

**Infusie vloeistof: Ringers lactaat met 1% humaan serum albumine*

Product alleen geschikt voor toediening bij deze patiënt
Alleen voor intraveneuze toediening
Identificeer nauwkeurig de bedoelde ontvanger van dit product
Product niet bestralen en geen leukocytenreductiefilter gebruiken
Uitsluitend voor gebruik binnen klinische trial
Investigators: Dr. A. van der Veldt / Dr. C. Lamers
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