Universitair Medisch Centrum
Utrecht

Individualized dosing of fludarabine during innate allo SCT
A randomized phase II study
*(**TARGET** Study)*

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Sponsor	**:**	**UMC Utrecht**
EudraCT number	**:**	**2018-000356-18**
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HOVON

HOVON associated study

PROTOCOL TITLE

Individualized dosing of fludarabine during innate allo SCT:

A randomized phase II study

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Short title	TARGET Study
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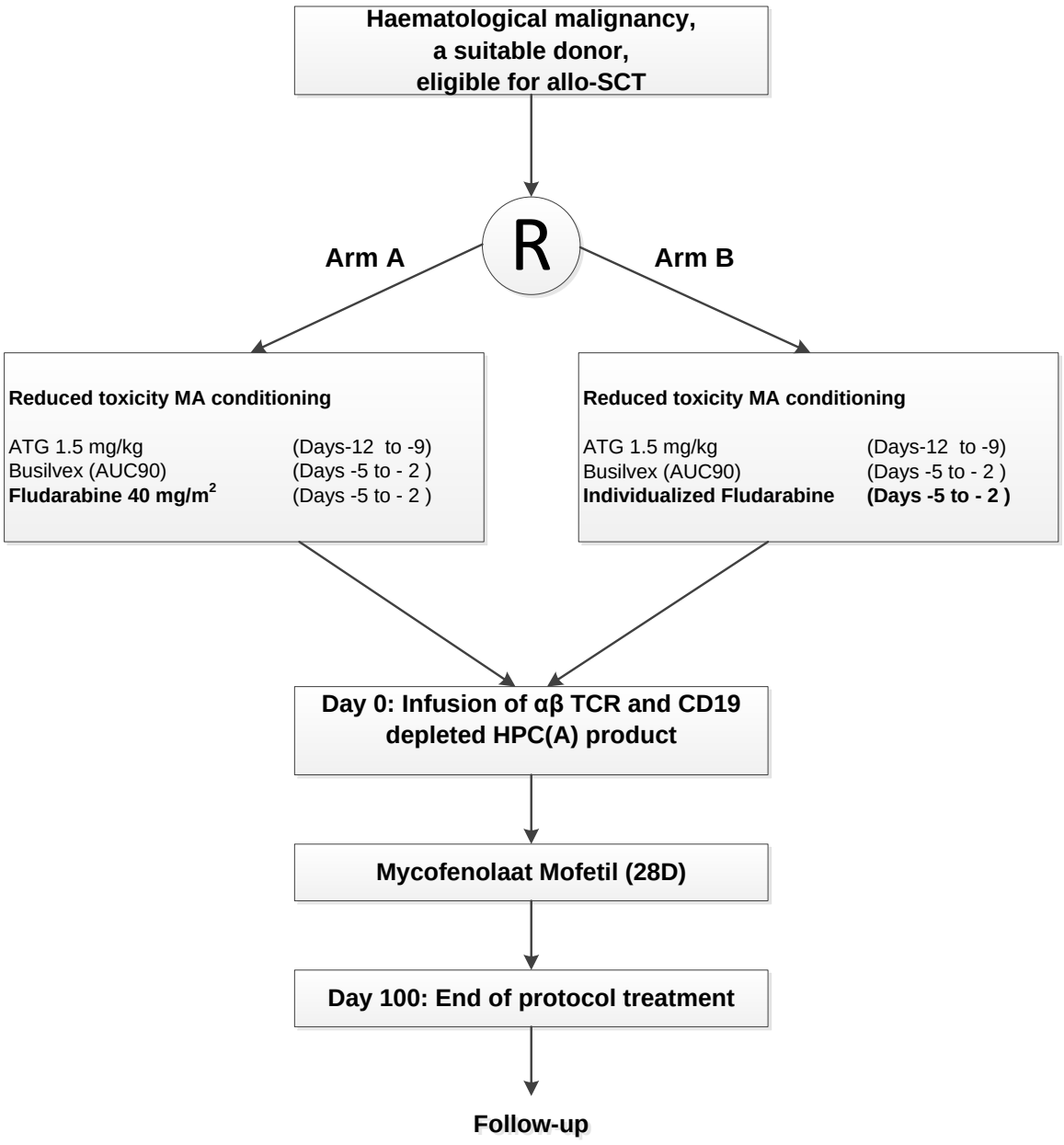
By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

LOCAL INVESTIGOR SIGNATURE SHEET

Local site name	
Local Investigator Name	
Local Investigator Signature	
Date	

By my signature, I agree to personally supervise the conduct of this study and to ensure its conduct in compliance with the protocol, informed consent, IRB/EC procedures, the Declaration of Helsinki, ICH Good Clinical Practices guideline, the EU directive Good Clinical Practice (2001-20-EG), and local regulations governing the conduct of clinical studies.

1. SCHEME OF STUDY



2. TABLE OF CONTENTS

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AdV	Adenovirus
AE	Adverse Event
aGVHD	Acute graft versus host disease
allo-SCT	Allogeneic hematopoietic stem cell transplantation
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
ANC	absolute neutrophil count
ATG	Anti-thymocyte globulin
AUC	Area under the curve
BMT-CTN	Bone Marrow Transplant Clinical Trials Network
BSA	Body surface area
Bu	Busulfan
CCMO	Central Committee on Research Involving Human Subjects
cGVHD	Chronic graft versus host disease
CI	Confidence Interval
CLL	Chronic lymphoid leukemia
CML	Chronic myeloid leukemia
CMV	Cytomegalovirus
CTCAE	Common Terminology Criteria for Adverse Events
DLI	Donor lymphocyte infusion
DPTP	Diphtheria-Pertussis-Tetanus-Polio vaccine
DSMB	Data Safety Monitoring Board
EBMT	European group for Blood and Bone Marrow Transplantation
EBV	Epstein Barr virus
EC	Ethics Committee
eCRF	Electronic Case Record File
EDC	Electronic data capture
EFS	Event free survival
Flu	Fludarabine
GMP	Good manufacturing practice
GVHD	Graft versus host disease
GVL	Graft versus leukemia effect
HHV 6	Human herpesvirus 6
HL	Hodgkin lymphoma
HPC (A)	Hematopoietic progenitor cell apheresis
HR	Hazard ratio
HSV	Herpes-simplex virus
ICH-GCP	International Conference on Harmonisation-Good Clinical Practice

IMP	Investigational Medicinal Product
IR	Immune reconstitution
i.v.	intravenously
MDS	Myelodysplastic Syndrome
MM	Multiple myeloma
MOF	Multiple organ failure
MPD	Myeloproliferative diseases
MRD	Matched related donor
MUD	Matched unrelated donor
NHL	Non Hodgkin Lymphoma
NK cells	Natural Killer cells
NFU	Nederlandse Federatie van Universitair Medische Centra
NRM	Non relapse mortality
OS	Overall survival
PBSC	Peripheral Blood Stem Cell
PD	Pharmacodynamic
PK	Pharmacokinetic
PTCy	Post-Transplant Cyclophosphamide
RI	Relapse incidence
SAE	Serious Adverse Event
SD	Selective decontamination
SoA	Schedule of Assessments
SOS	Sinusoidal obstruction syndrome
Sponsor	The sponsor is the party that commissions the organization or performance of the research, for example a pharmaceutical company, academic hospital, scientific organization 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 subsidizing party.
SUSAR	Suspected Unexpected Serious Adverse Reaction
TCR	T cell receptor
TDM	Therapeutic drug monitoring
VOD	Veno-occlusive disease
UMCU	University medical center utrecht
VR	Viral reactivation
VZV	Varicella zoster virus
WHO	World health organization
WMO	Medical Research Involving Human Subjects Act. In Dutch Wet Medisch-wetenschappelijk Onderzoek met Mensen

3. SYNOPSIS

Rationale: Allogeneic stem cell transplantation (allo-SCT) is still the treatment of choice for many patients suffering from hematological malignancies, which can only occasionally be cured with conventional chemotherapy. Donor T cells contribute strongly to the beneficial effect of allo-SCT due to a potent graft versus leukemia (GVL) effect after transplantation; however they also cause severe and life-threatening GVHD. In addition, relapses are frequently observed after allo-SCT. Recent reports have shown that the innate immune system can contribute to tumor control and control of infections, whereas the chance to induce GVHD appears to be low. Depletion of $\alpha\beta$ T-cells prior to allo-SCT is therefore a valuable tool of discarding the potentially harmful T cells. Many different studies now indicate that $\alpha\beta$ T-cell depletion in the graft reduces substantially life-threatening GVHD[1-5]. Also in the UMCU over 100 patients have received an αβT cell depleted allo-SCT. In the outcome analyses of the first 75 patients we confirmed the low incidence of GVHD as suggested by multiple other reports [1-5]. The cumulative incidence of severe III-IV aGVHD (0% at 3 months) and cGVHD (14%; 8% moderate/severe at 1Y) when utilizing an αβT cell depletion was markedly lower compared to our historical T cell replete cohorts. Low toxicity was also supported when analyzing the combined cumulative incidence of $\geq$ grade III viral reactivations and aGVHD II-IV, which was 47% at 6 months. Event free survival and overall survival were at least comparable to T cell replete transplantations. Thus, the major benefit of αβT cell depletion comes in the short run from the early window of opportunity to add additional immune interventions as well as in the long run from the very low incidence of cGVHD. However, analyzing the outcome of αβT cell depletion transplantation cohorts in depth also defined a group of patients who suffer from viral complications. Though the incidence of severe viral complications was low when compared to other cohorts, a retrospective analysis suggests that in particular patients with too high fludarabine exposures had an increased chance of profound infection. Current guidelines to adapt for fludarabine exposures seem thus to be suboptimal. Based on our retrospective analysis of T cell replete and T cell deplete transplantation cohorts, we developed an algorithm which should allow an easy and more individualized dosing of fludarabine resulting in an optimized and equivalent fludarabine exposure across all patients. We hypothesize that a more personalized dosing of fludarabine will translate into a lower incidence of severe viral infections, while low incidence of GVHD remains. This would render more patients eligible to early post allo-SCT interventions. In order to test this hypothesis we will randomize in this protocol the individualized dosing of fludarabine against standard of care arm, which does use dosages based on current guidelines.

Primary Objective: To address whether the individualized fludarabine conditioning reduces the incidence of severe viral infections at day 100 within the context of an αβTCR / CD19 depleted transplantation regimen.

Secondary Objective: To address whether the individualized fludarabine conditioning affects other clinical transplantation-related parameters, variations in individual fludarabine exposures and immunological reconstitution after an αβ TCR/CD19 depleted stem cell transplantation.

Study design: Prospective, multicenter, open label randomized, phase II study

Study population: Patients with a selected range of hematological malignancies eligible for allogeneic stem cell transplantation.

Intervention: Patients will be randomized to either to standard dosing of fludarabine or individualized fludarabine dosing as part of a conditioning regimen, followed by an αβTCR / CD19 depleted transplantation.

Target number of patients: 98 randomized

Expected duration of accrual: 2 years

Main study parameters/endpoints:

Primary Endpoint:

- Cumulative incidence of severe viral infections at day 100.

Secondary Endpoints:

- Non relapse mortality (NRM) at day 100
- aGVHD grade II-IV at day 100
- Donor engraftment (chimerism > 95%) at day 100
- Overall survival at day 100
- Cumulative incidence of relapse at day 100
- Effective fludarabine exposure

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

The protocol comprises a different dosing of fludarabine in the experimental arm. All other acts, measurements, follow-up and level of care are therefore similar to off-study patients undergoing allo-SCT. The burden of the therapy is associated with the allo-SCT itself, which is a necessary therapeutic intervention in all subjects. Possible increased risks for the recipient are graft failures, though not observed so far in all cohorts with the intended dose levels. The intended target level of fludarabine remains in the range of all so far treated patients at the UMCU. We only propose to avoid too high exposure to fludarabine. Possible benefits include a proposed lower incidence of infections, low incidence of GVHD as well as optimized cancer surveillance due to a more balanced immune reconstitution.

DSMB and safety reporting: A DSMB will be installed which will advise the investigators about (dis)continuation of the trial during the study. An yearly report will be presented, which includes data on overall survival and non-relapse mortality.

4. INTRODUCTION AND RATIONALE

Allogeneic stem cell transplantation (allo-SCT) is until today the most effective immunotherapy for hematological malignancies and could not be replaced by the majority of drugs entering the market [6]. However, transplant related mortality caused by GVHD and infections is still reported in up to 40 % of patients. In addition – depending on the underlying disease – also relapses frequently occur. Thus there is an urgent medical need to further improve outcomes after allo-SCT. To date allo-SCT is no longer considered as “one shot intervention” and two basic pillars can be distinguished. A first pillar consists of conditioning regimen, graft properties and immune suppression. The second pillar is maintenance therapy usually starting around day 100 with e.g. DLIs [7, 8]. Studying both pillars separately will allow segregating the major challenge of acute toxicities until day 100 as well as maintenance questions for the long term. Creating a next generation of complementary studies separating these two pillars will thereby generate an opportunity to rapidly test different interventions meant to further reduced toxicity of allo-SCT. In addition, this strategy creates the unique opportunity to allow substantial improvements and harmonization’s of the field. This creative change in study designs has also been picked up most recently e.g. by the FDA in order to allow swift changes in clinical practice in fields of urgent medical need in an era where many different drugs are competing for market approval and positioning [9].

Others and we gained through the last years vast experience in graft engineering through $\alpha\beta$ T cell depletion ([1-5] and section 4.1), which aims to guide more patients to early additional immune interventions after allo-SCT. αβ T cell depletion results in lower incidences of aGVHD as compared to T cell replete allo-SCT regimens. As the first 100 days after allo-SCT are also prime time for complications such as viral infections, evaluating the clinical impact of changes in the first pillar on viral infections at day 100 becomes for many clinical trials a key question. The primary gain of this study is therefore to reduce the cumulative incidence of severe viral infections at day 100 in order to increase the number of patients who will be eligible for later interventions early after transplantation [10, 11]. Within this context we will study whether a further individualized transplantation regimen is able to eliminate inter individual variations in drug levels and consequently complications after allo-SCT. We will focus on a more refined application of the drug fludarabine and compare this to standard dosing of fludarabine in order to develop in the era of precision medicine a personalized transplantation platform aimed to guide more patients to maintenance therapies.

4.1 The αβT cell depletion platform

4.1.1 Background

αβ T-cells play a crucial role in the pathology of aGVHD – especially shortly post allo-SCT when inflammation is induced by pretreatment of the recipient[12]. Therefore, major developments in novel transplantation strategies include alternative targeting and dosing of αβ T-cells during and after allo-SCT. High dose cyclophosphamide has been shown to preferentially target proliferating, alloreactive T cells[13], which is currently increasingly used as immune prophylaxis post transplantation (PTCy)[14]. However, personalizing cyclophosphamide in contrast to e.g. busulfan during and after allo-SCT is a major challenge [15] limiting thereby the potential of cyclophosphamide for personalized medicine.
Alternatively, a stringent *in vivo* and *ex vivo* T cell depletion has since long been known as an effective strategy to prevent severe GVHD[16]. It has recently been shown in T cell replete transplantation that one log increase in T cell numbers can significantly increase the risk of developing GVHD[17]. *In vivo* depletion of T cells has been extensively explored with the addition of ATG. A major development are current efforts to evaluate levels of ATG both pre- and post-allo-SCT[18, 19] , as well as the development of prediction models to better master large individual variations in pharmacokinetics (PK) and pharmacodynamics (PD)[20].
Alternatively, *ex vivo* T cell depletion has been recognized as an effective strategy to prevent GVHD[16]. Alemtuzumab is being used with success in matched related donors (MRD) and matched unrelated donors (MUD)[16]. However, a variety of approaches have been reported, such as *in vivo* and '*in vitro*-in the bag' T cell depletion methods indicating the lacking consensus and standardization of this method. More recently, both CD34 selection[11, 21] and αβ TCR depletion with the antibody based depletion method developed by Miltenyi Biotec ® successfully entered clinical practice in haplo-SCT and for matched related and matched unrelated donors (MRD and MUD) ([1-5]; review in[22, 23] and section 4.1.2).

4.1.2 Dutch experience with the αβT cell depletion platform

In the UMCU 75 patients with acute leukemia, MDS, MPN or lymphoma's received an allo - SCT conditioned with ATG, Busulfan and Fludarabine followed by 4 weeks of MMF, as also outlined study arm A, followed by infusion of an αβT cell depleted graft. Data of 54 patients were retrospectively analyzed, data of 21 patients were prospectively collected and analyzed (Table 1, 2).

Median age (range)	54 (19-73)
Female gender (%)	30 (42)
Median time post allo-HSCT (m)	12 (0-33)
AML*	**24 (32%)**
CR 1 (%)	18 (24)
> CR1 (%)	4 (5)
Relapsed/refractory (%)	2 (3)
ALL	**12 (17%)**
CR 1 (%)	6 (8)
> CR1 (%)	3 (4)
Relapsed/refractory (%)	3 (4)
MDS^	**14 (19%)**
Untreated (%)	6 (8)
CR 1 (%)	5 (7)
> CR1 (%)	3 (4)
NHL/HD#	**15 (20%)**
> CR1 (%)	7 (9)
SD/PD (%)	8 (11)
MPN	**7 (9%)**
CR (%)	1 (1)
SD/PD (%)	6 (8)
Other~	**3 (4%)**
Donor type	
MRD	19 (25)
10/10 MUD	52 (69)
9/10 MUD	11 (15)
CMV donor/recipient	
+/+	23 (31)
+/-	10 (13)
-/+	16 (21)
-/-	31 (41)
EBV donor/recipient	
+/+	58 (77)
+/-	7 (9)
-/+	11 (15)
-/-	1 (1)
not evaluated	3 (4)

* Favourable cytogenetics n=1; Intermediate cytogenetics n=8; adverse cytogenetics n= 17

^ Intermediate cytonetics n= 10; adverse cytonetics n=10

Indolent B-NHL n=2; MCL n=3; T cell NHL n=2; HD n =3; DLBCL/Transformed B-NHL n=5

~ plasmacelleukemia n=2; CML blast crisis n=3

Table 1: Patient characteristics: Retrospective cohort

Median age (range)		59 (19-69)
Female gender (%)		6 (29)
Median time post allo-HSCT (m)		5 (1-11)
AML*	**8 (38%)**	
CR 1 (%)		4 (19)
> CR1 (%)		1 (5)
3 (14)		1 (5)
ALL	**3 (14%)**	
CR 1 (%)		1 (5)
Relapsed/refractory (%)		2 (10)
MDS^	**4 (19%)**	
Untreated (%)		3 (14)
CR 1 (%)		1 (5)
NHL/HD#	**1(5)**	
SD/PD (%)		1 (5)
MPN	**3 (14%)**	
SD/PD (%)		3 (14)
Other~	**2 (10%)**	
Donor type		
MRD		3 (14)
10/10 MUD		13 (62)
9/10 MUD		5(24)
CMV donor/recipient		
+/+		5 (24)
+/-		2 (10)
-/+		5 (24)
-/-		9 (42)
EBV donor/recipient		
+/+		16 (76)
-/+		3 (14)
not evaluated		2 (10)

* Intermediate cytogenetics n=2; adverse cytogenetics n= 6
^ Intermediate cytonetics n= 2; adverse cytonetics n=2
# DLBCL/Transformed B-NHL n=1
~ plasmacelleukemia n=2

Table 2 Patient characteristics: Prospective cohort.

No graft failures have been observed. The incidence of aGVHD grade III-IV at day 100 was 5.6% (n=1) in prospective cohort and 0% in retrospective cohort (data not shown). In the combined retrospective and prospective cohort, the CI of aGVHD II-IV is 17% at day 100, the CI of viral infections > grade III was 38% at day 100 and the combined CI of aGVHD grade II-IV and viral infections $\geq$ grade III was 47% at day 100 (Figure 1). Amongst the viral grade III infections, 2 cases of PTLD (2.5%) were observed.

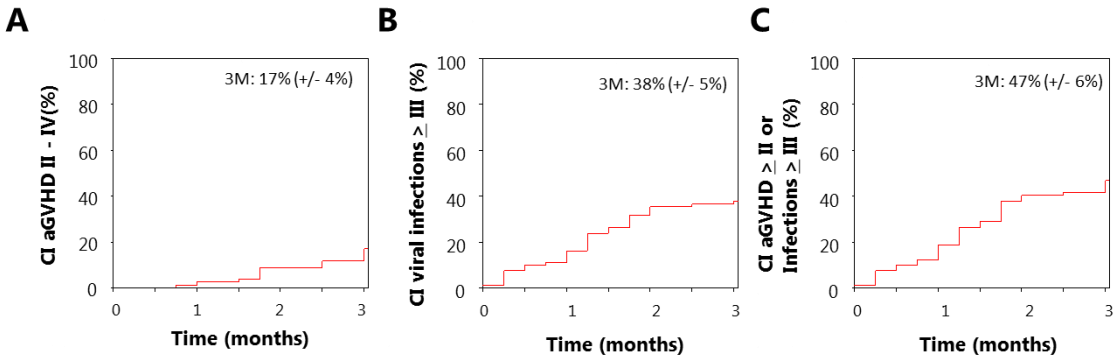


Figure 1: CI of grade II-IV aGVHD (A), viral infections ≥ grade III (B) and combined CI of aGVHD II-IV or viral infections ≥ grade III (C). Death and relapse are competing events. Pooled data of retrospective and prospective cohort (n=75). Viral infections can be either CMV, EBV or BK. Grading is performed according to CTC-AE V4.0. Grade III implies either an infection requiring i.v. medication or admission to the hospital.

Efficacy was analyzed in the retrospective cohort, given the short follow-up of the prospective cohort. With a median follow-up of 13 months, the 2-year (2Y) event free survival (EFS) was estimated to be 61% and the overall survival (OS) to be 65%. The cumulative relapse incidence was 25% and the NRM 14% (Figure 2). Interestingly, long term survivors had a very low incidence of cGVHD despite multiple DLIs, suggesting that this platform does not only allow early immune interventions but also associates with a rather low long-term toxicity (Figure 3A).

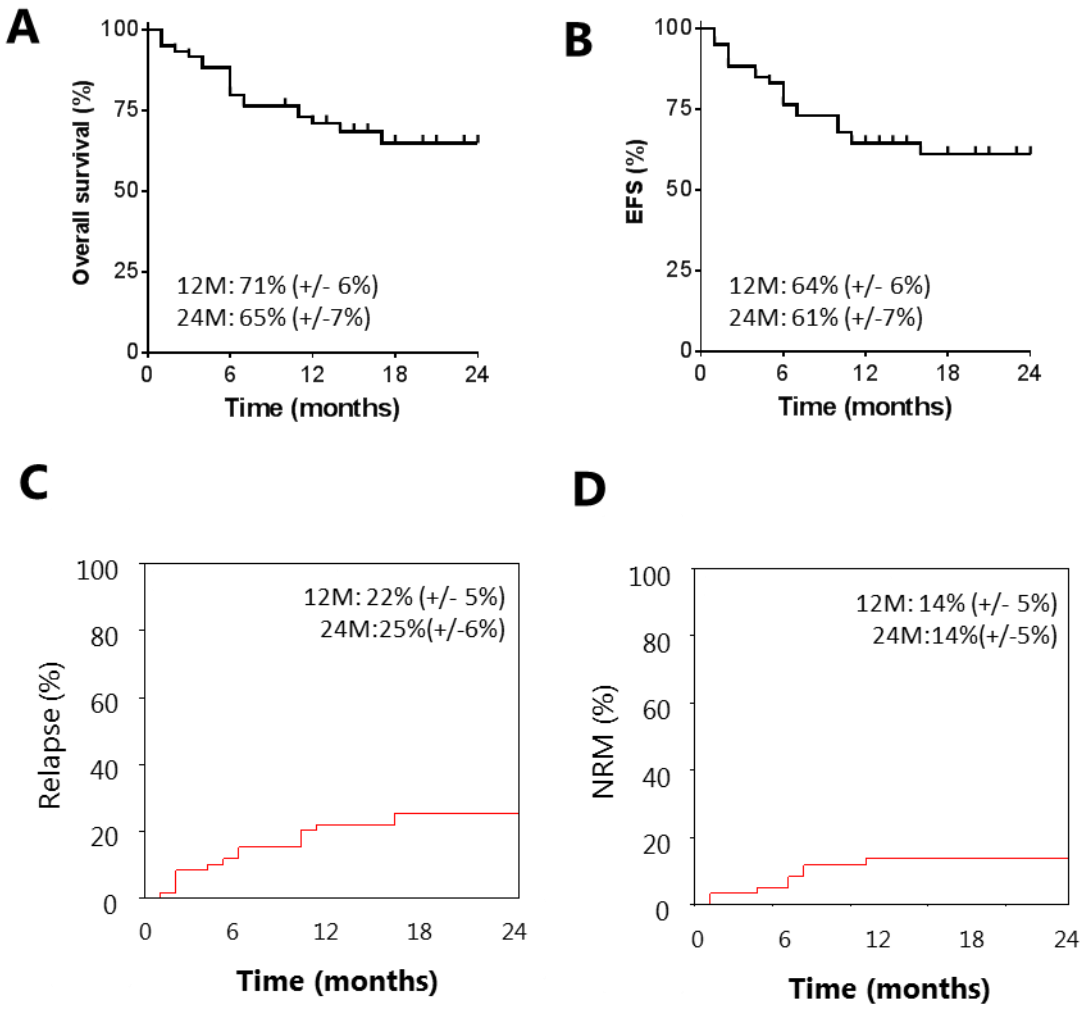
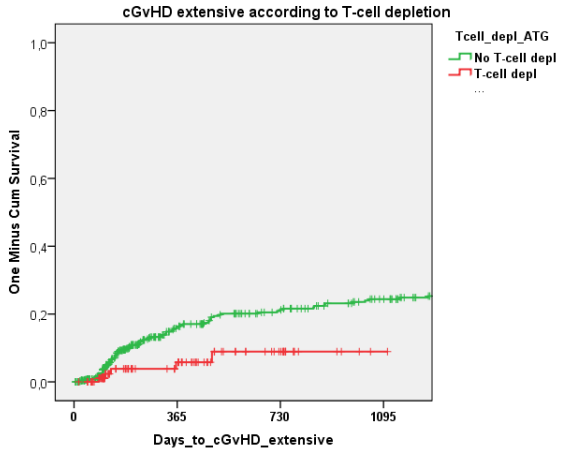


Figure 2: Overall survival (A), EFS (B), cumulative incidence of relapse (C) and NRM (D) of retrospective cohort. Cause of NRM see below.

The main cause of NRM was analyzed in all patients. GVHD (induced by a 2nd DLI because of mixed chimerism) contributed to 1 death (8%). This is in contrast to an internal historical T cell replete control cohort, where GVHD was the main cause of death in > 60% of the patients (data not shown). Infections were the main cause of death in 54% (n=6) of the patients and included viral, bacterial and fungal infections (Figure 3B). Two patients died of multi organ failure (MOF) not directly related to infections and/or GVHD. The causes of dead classified as 'other' include intestinal ischemia during conditioning and cerebral bleeding shortly post allo-SCT; GVHD grade II (esophagitis) with cachexia, after which patient refrained from further supportive care; multiple infections after which patient decided to stop treatment.

A



B

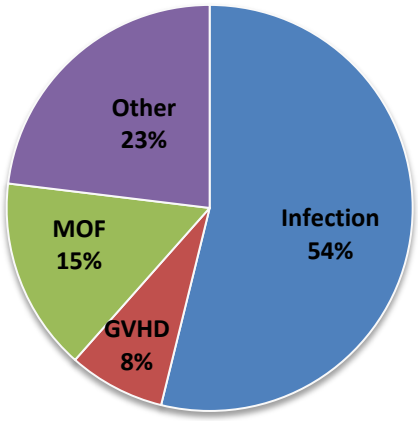


Figure 3: A) Incidence of cGVHD. (B) Main cause of death for patients who did not die as a consequence of the relapse of the disease (n=13). 'Other' is explained in main text.

4.2 Targeted conditioning

Busulfan (Bu) combined with Fludarabine (Flu) and ATG is a frequently used conditioning regimen in allogeneic hematopoietic cell transplantation (allo-SCT). For Bu and ATG (Thymoglobuline), optimal exposures in relation to survival have recently been described for paediatric and adult patients[18]. For Flu these data are sparse. International recommendations for dosing fludarabine are currently based on the creatinine clearance, mainly meant to prevent severe pancytopenia e.g. within the context of CLL therapies. Also in allo-SCT, data indicate that dose adjustments of fludarabine might be beneficial on the outcome of allo-SCT[24]. However, recent analysis done in the UMCU (section 9.2) indicate that these recommendations are not sufficient to cover real-life fludarabine levels within the context of allo-SCT. In summary, our retrospective analyses indicates that a personalized fludarabine exposure reduces toxicity and NRM, resulting in an improved OS.
Personalized dosing could be either achieved by determining levels and then adjusting doses as e.g. suggested for busulfan. Alternatively, easier methods like dosing on clinical parameters would allow a broader use in daily clinical practice.

In the context of αβTCR / CD19 depleted allo-SCT we hypothesize that a reduction of overexposure to fludarabine results in improved immuno reconstitution (IR) and a reduction of early toxicity, especially viral infections. In this study we will prospectively test if

personalized fludarabine results in a reduced incidence of severe viral infections at day 100 compared to recipients which are treated according to the control arm.

4.3 Conclusions

Collectively, αβ T-cell depletion of PBSCs derived from MRD/MUD results in a significant reduction of grade III aGVHD (figure 1). Also the incidence of cGHVD appears to be low (figure 3 left), although longer follow-up is warranted. The main cause of transplant related toxicity and morbidity is determined by infections (figure 3 right).

Overexposure with fludarabine associates with an increased incidence of viral infections (see below, section 9.2.4.). We hypothesize that by individualized dosing with fludarabin the change of overexposure decreases. As a consequence, we expect a reduction in the incidence of severe viral infections, a decrease in NRM and an increase in Quality of Life.

5. OBJECTIVE

5.1 Primary endpoint:

- Cumulative incidence of severe viral infections (≥ grade 2 as in APPENDIX E) until day 100

5.2 Secondary Endpoints:

- Engraftment
    - Time to neutrophil engraftment
    - Time to platelet engraftment
    - Donor engraftment (chimerism > 95%) at day 100
- Clinical outcome
    - aGVHD until day 100
    - NRM until day 100
    - Number of graft failures until day 100
    - Event free survival (EFS: i.e. time from transplantation until progression/relapse, graftfailure or death from any cause, whichever comes first)
    - Overall survival (OS) calculated from transplantation. Patients still alive or lost to follow up are censored at the date they were last known to be alive
      Incidence of infections
    - Incidence and grade of chronic GvHD

  - Long term NRM (1Y)
  - Long term (secondary) graft failure (1Y)
- PK/PD
  - ATG exposure
  - Fludarabine exposure
  - Busulfan exposure
- Immunoreconstitution
  - Immune reconstitution including but not limited to total number of $CD3^+$ T cells, $CD4^+$ and CD8+ subtyping of T cells, CD3-CD16/56+ (NK cells), γδT-cells at 3, 6, 12 and 24 months after transplantation, assessment of NK and TCR repertoires at defined time points with personalized fludarabine conditioning.
  - Graft composition (CD34+ cells, αβ T cells, γδ T cells, NK cells, B cells)

6. STUDY DESIGN

This is a multicenter prospective, open label randomized, phase II study (UMCU (Utrecht), LUMC (Leiden), Johannes Gutenberg University (Mainz)). Patients with hematological malignancies according to the inclusion criteria with a matched related donor or a matched unrelated donor, are treated with a αβTCR / CD19 depleted allo-SCT. Conditioning regimes are depicted on 8.3 which differ only in:

Arm A: Fludarabine based on classical dosing
Arm B: Individualized fludarabine aimed to avoid overexposure (section 9)

All other interventions, except for alternate dosing of fludarabine in arm B, are considered as standard of care. All subjects will complete the end of treatment visit at day 100. After day 100, subjects will enter an extended follow-up of 1 year.

7. STUDY POPULATION

The study population includes patients with hematological malignancies according to the inclusion criteria, eligible for allo-SCT and a suitable donor. Inclusion of 98 patients within 2 years should be feasible.

7.1 Inclusion criteria

1. Adults (≥ 18 years)
2. AML, MDS, ALL, CML, CLL, NHL, HL, or a myeloproliferative disease (MPD)
3. Indication for allo-SCT according to the policy of the local center
4. WHO performance status ≤ 2
5. Written informed consent

7.2 Exclusion criteria

1. Relapse of disease within 5 months after previous allo-SCT
2. Bilirubin and/or transaminases > 2.5 x normal value*
3. Creatinine clearance < 40 ml/min*
4. Cardiac dysfunction as defined by:
    - Unstable angina or unstable cardiac arrhythmias
    - NYHA classification > II (Appendix B)
    - Cardiac symptoms and/or history of cardiac disease AND a cardiac ejection fraction < 45%
5. Active, uncontrolled infection

***Assessed < 2 weeks prior to allo-SCT*

8. TREATMENT OF SUBJECTS

8.1 Donor selection

Either HLA matched siblings (MRD) or matched HLA matched unrelated donors (MUD) will be eligible (9/10 or 10/10). Donor selection will be performed in line with local guidelines.

8.2 Stem cell collection

Donors will be treated with recombinant human granulocyte colony stimulating factor at a dose of 10 microgram/kg/daily subcutaneously divided in 2 doses for 5 days. Leukapheresis will be undertaken at day 5. The aimed cell number for collection is between 5-10 x 10^6 and infusion between 2-10 $CD34^+$ cells/kg.

8.3 Conditioning regimen

Treatment arm	Drug	Dose	Days
A+B	ATG (Thymoglobulin ®)^	1.5 mg/kg/d (total 6mg/kg)	-12 to -9
A+B	Busulfan (Busilvex ®)*	(AUC90)	-5 to -2
A	**Fludarabin (standard)**	40mg/m^2/d (total 160 mg/m^2)	-5 to -2
B	**Individualized fludarabin (TDM) ****	See below	-5 to -2

^For prevention of serum sickness / as anti-emetic steroids will be adminitered according to the follow guideline: prednisolon will be administered: 100 mg i.v. day -13 to -9; prednisolon 20 mg p.o day -8-6; dexamethason i.v. day -5 to -2; prednisolon 10 mg p.o. day -1 to day 0; prednisolon 5 mg day 1

* **Busilvex** will be administered intravenously for 4 days in 180 minutes and will be prepared by the pharmacy. Therapeutic Drug Monitoring of busilvex will be performed and adjusted / targeted to a cumulative AUC of 90mg*h/L (+/-10%) to reach a situation of myeloablation and limited toxicity. i.v. busilvex with AUC monitoring can be replaced by i.v. busilvex (3.2 mg/kg/day at day -5 to day -2 without required monitoring) or with oral busulfan (1 mg/kg a 6 hours day -5 to -2) according to the discretion of the local investigator.

** **Fludarabine** will be administered intravenously for 4 days (days -5 to -2) in 60 minutes and will be prepared by the pharmacy . In arm B dosing of fludarabine will be individualized by using therapeutic drug monitoring (TDM) targeted to a cumulative AUC range of 15-25 mg*h/L. The protocol regarding TDM can be found at: https://www.umcutrecht.nl/nl/Ziekenhuis/Professionals/Diagnostiek-aanvragen/Farmalab.
In case TDM is not available, individualized fludarabine will be based on BSA and renal function according to formula 1# .

Individualized dosing formula fludarabine:

$$\text{Cumulative dose} = 20\ \text{mg} * \text{h/L} * \frac{365\ \text{g}*\text{mol}^{-1}}{285\ \text{g}*\text{mol}^{-1}} \left(0.782 * \text{eGFR} * \frac{70\ \text{kg}*\text{BSA}}{1.73\ \text{m}^2*\text{BW}} * \frac{1000\ \text{ml/L}}{60\ \text{min/h}} + 3.24\ \text{L/h}\right) * \frac{\text{BW}^{0.75}}{70}$$

where the cumulative dose is in mg fludarabine-phosphate, eGFR is in ml/min/1.73m^2, BSA is in m^2 and BW is in kg[25].

8.4 HPC(A) products

Depletion of αβTCR/CD19+ lymphocytes will be performed with anti-αβTCR and anti-CD19 antibodies in combination with magnetic microbeads, using the automated CliniMACS device

(Miltenyi Biotec, Bergisch Gladbach, Germany). The cell depletion method is an established procedure in an allo-SCT setting [1-5, 23] and will be performed according to standard operating procedures. The number of T cells, B cells, and the T-cell subsets will be measured in the graft. The aimed maximal contamination with $\alpha\beta$ T-cells is $5x10^5$/kg. The aimed maximal contamination with B-cells is $1x10^5$ $CD20^+$-cells.

In case of a major ABO-incompatibility between patient and donor and in case when the number of red cells exceeds 200 x 10^9 in the end product, HPC(A) will be infused slowly according to the local guidelines. All HPC(A) products will be infused within 24 hours after the depletion procedure. Grafts will be infused at day 0.

8.5 Management of graft failure

Primary or secondary graft failure as judged by neutrophil counts will be considered a treatment failure. Patients will be treated according to institutional guidelines at the investigator's discretion. Management of graft failure should be discussed with the national coordinating investigator or the respective leading investigator.

8.6 GVHD prophylaxis

MMF 15mg/kg 3 times a day (max 1000 mg 3 times a day) will be administered for 28 days as standard GHVD prophylaxis. If the contamination with $\alpha\beta$ T cells is above $5x10^5$ $\alpha\beta$ T cells / kg, full immune prophylaxis will be administered according to the local guidelines. If the contamination with B cells is above 1x10^5 B cells / kg, no additional immune prophylaxis needs to be administered.

8.7 Treatment of GVHD

Treatment of GVHD will be performed according to the guidelines of the local investigator.

8.8 Infection prophylaxis

Infections should be controlled before start of the conditioning regimen according to local guidelines. Selective decontamination (SD) consisting e.g. of anti-bacterial agents and antimycotic agents will be administered according to local protocols. Surveillance cultures will be sampled according to local protocols. Monitoring, prophylaxis and treatment of viral infections (e.g. CMV and EBV) will be performed according to local protocols. Pneumocystis carinii and toxoplasmosis prophylaxis is administered according to local protocols. Vaccination (e.g. Pneumovax/DPTP/influenza) is administered according to local guidelines.

8.9 Other required co-medication

For prevention of serumsicknes and as anti emetics steroids are administered as follows:

- days -13 to -9 prednisolon 100 mg i.v.;
- days -8 to -6 prednisolon 20 mg p.o.;
- days -5 to -2 dexamethason 10 mg i.v.;
- days -1 and 0 prednisolon 10 mg p.o.;
- day 1 prednisolon 5 mg p.o.

To reduce the chance of development of hepatic complications such as hepatic aGVHD or 'Sinusoidal obstruction syndrome or veno-occlusive disease' (SOS/VOD), administration of ursodeoxycholic acid (300 mg two time a day; starting at the first day of the Bu/Flu conditioning until 3 months after allo-SCT) is allowed[26].

9. INVESTIGATIONAL MEDICINAL PRODUCT: Fludarabine

9.1 Summary of findings from non-clinical studies

Fludarabin is registered for treatment of chronic lymphatic leukemia (CLL). Although not officialy registered Fludarabin is utilized worldwide as part of conditioning regimens for allo-SCT for decades[27, 28] as well as in our center[29].

The defintion of the investigational product (which implies an alternative dosing regemin of fludarabin) is based on retrospective data of patients who underwent an allo-SCT in the UMCU (section 9.2).

9.2 Summary of findings from clinical studies

In the UMCU we retrospectively analyzed 192 patients (73 children, 119 adults; 124 malignant and 68 benign disorders) patients transplanted between 2010 and 2016, after myeloablative conditioning with busulfan (targeted to an area under the curve of 90 mg*h/L), fludarabine 160mg/m^2 and ATG. The optimal fludarabine exposure was derived through relating cumulative fludarabine exposure to outcomes: event-free survival (EFS), graft-failure, relapse and non-relapse mortality (NRM). Subsequently, the effect of cumulative fludarabine exposure was evaluated on EFS, overall survival (OS), NRM, relapse, immune reconstitution and viral reactivation. Finally, different dosing strategies were compared in their ability to achieve optimal target attainment. Parametric time-to-event models, Cox proportional hazard models and Fine-Gray competing risk models were applied.

9.2.1 Pharmacokinetic analyses and exposure optimum

A population PK model for Flu was developed that accurately described concentration versus time data and was extensively evaluated. In the model, bodyweight and renal function were

shown to be predictors for Flu clearance[30, 31]. Flu exposure measures could be accurately estimated for all patients using the validated population PK model. Cumulative exposure to Flu for all doses ($AUCT_{0-\infty}$) was shown to be the best predictor for EFS (Supplemental results: p7). On the high end of Flu exposure, the incidence of NRM increased (p<0.001, Figure 4B) and at lower exposures more graft failures were observed (p=0.03, Figure 4C). Flu exposure had no significant influence on relapse (p=0.88, Figure 4D). This resulted in a minimal event probability at a cumulative Flu exposure of 20 mg*h/L (15-25 mg*h/l, Figure 4A). The exposure target was found the same among different ages and indications.

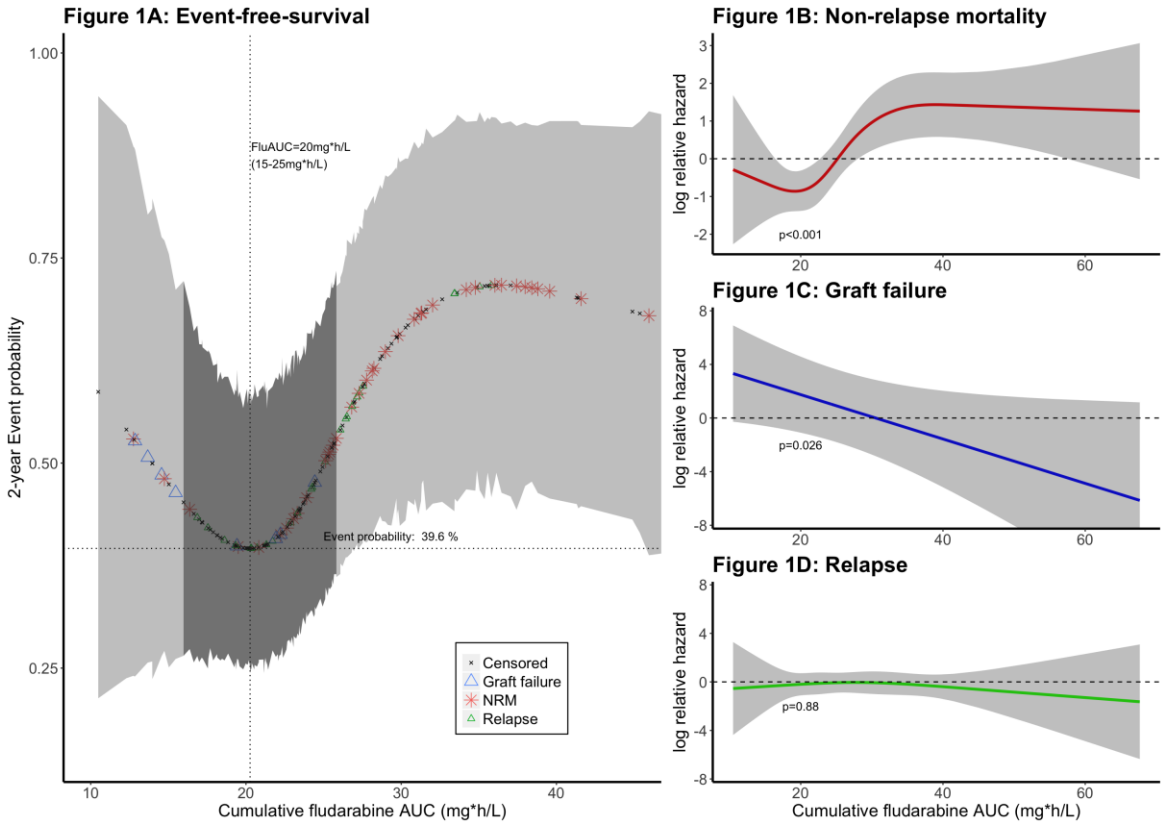


Figure 4: Relating Flu exposure to different outcomes. A) composite events: symbols correspond to the estimated event probability (y-axis) of sequential patients at their cumulative fludarabine AUC (x-axis). Red stars indicate the occurrence of NRM, blue triangles indicate GF, green triangles indicate relapses and the black stars correspond to patients without events. The shaded area depicts 95% CI and the dark shaded area corresponds to the established target area. Figure B-D: lines depict the estimated logarithm of the hazard ratio (y-axis) at the given fludarabine AUC (x-axis), for B) NRM C) GF D) Relapse. Shaded areas depict 95% CI. Displayed hazards (B-D) and event probability (A) correspond to a patient at the median age of 35 years, diagnosed with Leukemia/Lymphoma and no prior HCT. P-values (figure A-D) are calculated by likelihood ratio test using backwards deletion from the full regression model.

9.2.2 Main outcomes

The 2-year EFS probability for the optimal exposure group (65%, 95% CI 55-76%) was significantly higher compared to the above-optimum-group (32%, 16-59%, p<0.001; Hazard ratio [HR] 1.9, 95% confidence interval [CI] 1.1-3.2, p=0.027; Figure 5A). Trends were similar for OS compared to EFS (Figure 5B): OS in the optimally exposed group was 66% (55-78%) compared to (43%, 95% CI: 29-64%, p<0.001) in the above-optimum-group (HR: 2.1, 95% CI: 1.2-3.7, p=0.015).

The lower EFS and OS in the above-optimum-group was caused by a higher incidence of NRM (HR: 3.1, 95% CI: 1.5-6.3, p=0.002; Figure 5C) and no difference in relapse (p=0.54, Figure 5D). In addition, the risk for graft failure and NRM were increased in the below-optimum-group (HR 3.4, 95% CI 1.2-9.6, p=0.022; Figure 5C and HR 4.3, 95% CI 0.98-19, p=0.054; Figure 5E respectively). No graft-failures were observed in the above-optimum-group.

IR was significantly lower in patients exposed above optimum, with a decrease in 100-day IR from 57% (46-58%) after optimal Flu exposure to 33% (19-47%) after exposure above optimum (p=0.001). Viral reactivation (VR) followed the same trend with an increased incidence VR from 6% to 25% (optimal vs. above-optimum, p=0.009). Notably, in both IR (as found in literature[32-34] and VR there was a significant effect of age (p<0.001 and p=0.007 respectively). By adjusting for age the hazard for VR decreased for patients exposed above optimum (HR: 1.7, 95% CI: 0.77-3.8, p=0.2), but not for IR (HR: 0.59, 95% CI 0.35-0.92, p=0.018). Sub-group analyses were performed for pediatrics (age≤20), adults (age>20), and patients receiving an $\alpha\beta$-T cell depleted graft. A similar trend regarding OS and EFS was found for all three groups (data not shown).

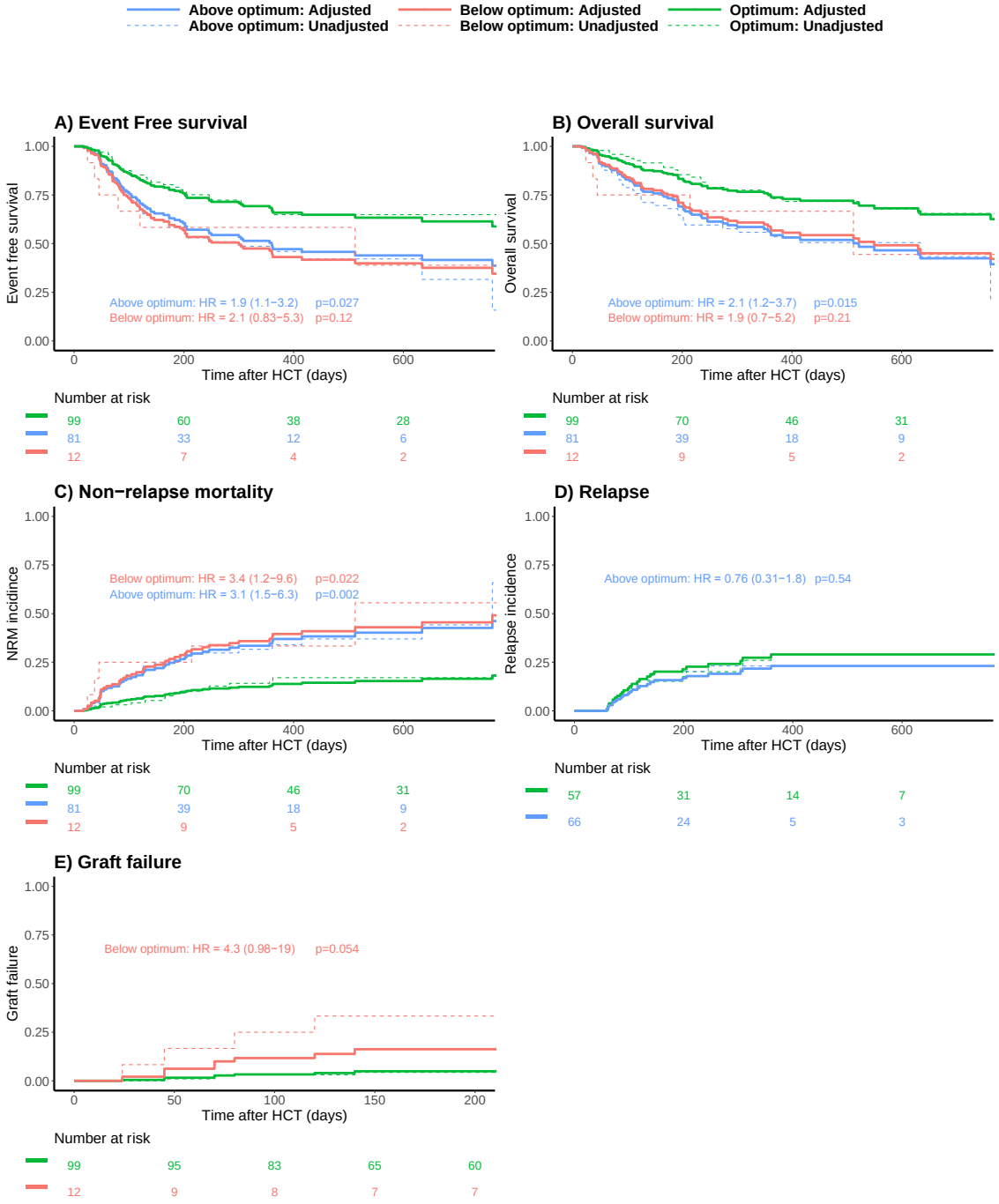


Figure 5: Data are adjusted (solid lines) and unadjusted (dashed lines) estimations of, (A) event-free survival, (B) overall survival, (C) non-relapse mortality, and (D) relapse (only patients with malignant disease), and (E) Graft failure. Adjusted estimations are to be interpreted as the expected outcomes if all exposure groups were the same as the average of the full cohort, with respect to all multivariate predictors (age, diagnosis, prior allo-SCT). P-values are derived from the Wald's test in the full regression model. There was 1 patient with a malignancy exposed below optimum and no graft failures were observed above optimum, hence these groups are not shown.

9.2.3 Dosing regimens

Plasma samples routinely obtained for Bu TDM were stored (-80˚C) until quantification of Flu using a validated liquid chromatography mass spectrometry method.[35] Subsequently, a population PK model was developed and validated to describe Flu pharmacokinetics and to identify patient characteristics influencing variability between patients. The developed model was used to estimate measures of Flu exposure: the cumulative area under the plasma concentration-time curve (AUC) from start of conditioning ($AUCT_{0-\infty}$) and the post transplantation AUC ($AUC_{Tx-\infty}$). The two Flu exposure measures were quantitatively linked to the primary outcome measure (2-year event free survival; EFS) using a parametric time-to-event model. We selected the PK exposure measure showing the strongest relationship with the event hazard, as quantified by means of the Akaike information criterion[36]. Subsequently, the selected model was used to plot 2-year event probability (1-EFS) versus Flu exposure. We then identified a target exposure window, by taking the exposure congruent with minimal event probability and expanding it to +/- 25%, thus defining an optimal exposure group. After identification of the target exposure area, different dosing regimens and algorithms were assessed in their ability to attain this target. For this, we compared estimated exposures after three different dosing algorithms: 1) the current dosing regimen, 2) dosing based on only the newly developed Flu population PK model and 3) the current dosing combined with therapeutic drug monitoring (TDM) based on measured drug concentrations on day 1.

Simulated exposures for all three dosing regimens in the full cohort, pediatric subset (≤20 years, n=68) and adult subset (>20 years, n=118) are depicted in Figure 6A, 6B and 6C respectively. PK target attainment was low using the current dosing regimen of 160 mg/m2 (50%), particularly in the adult population (42%): caused by a high percentage of exposures above optimum (58%). The dosing regimen based on the developed PK model increased target attainment to 77%, mainly by reducing high exposures in both adults and children (reduction of 40% and 18%, respectively), although a slight increase in exposures below optimum was observed (6% increase). TDM performed best with 99% of patients reaching the target exposure.

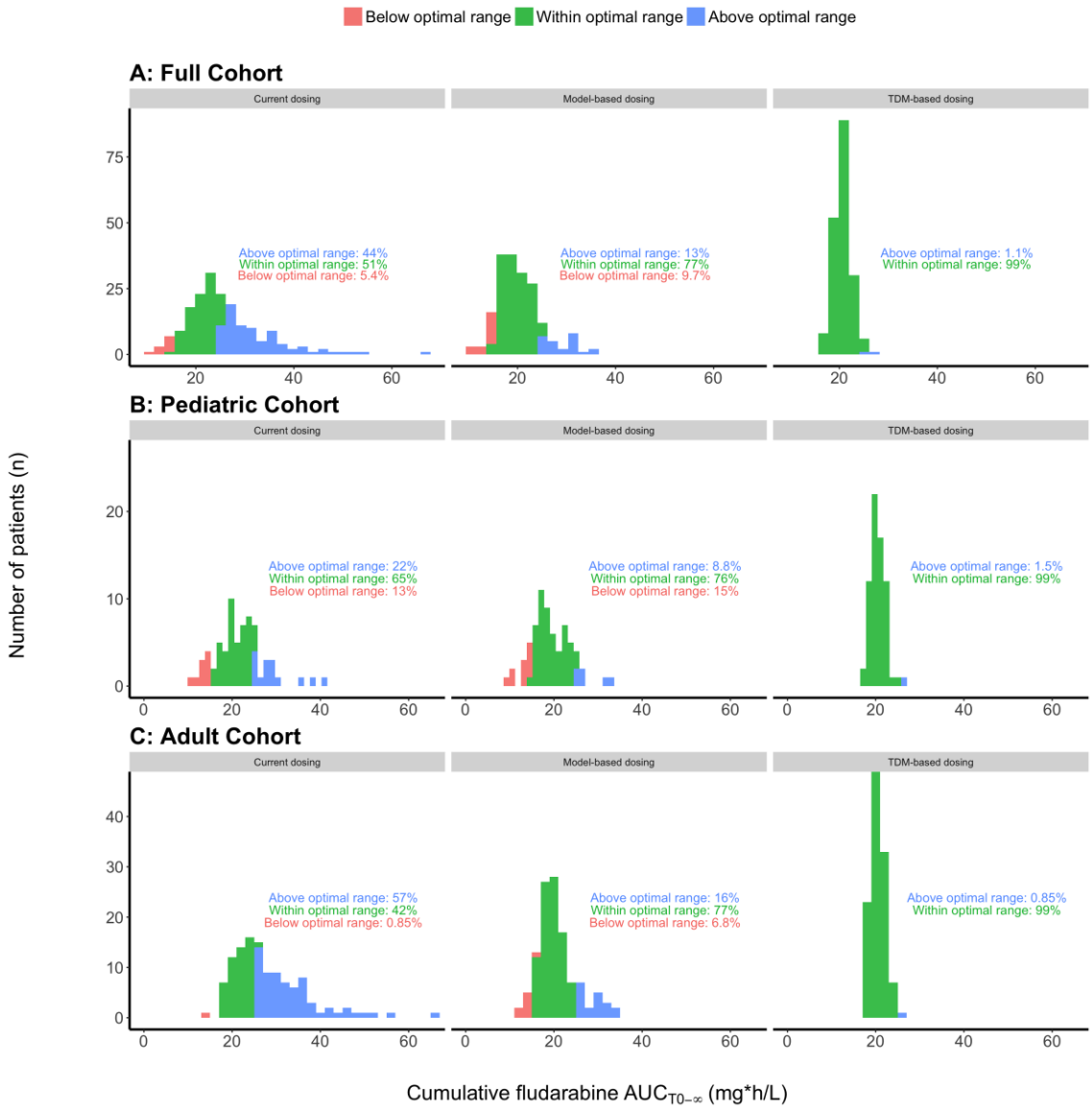


Figure 6: Comparing dosing regimens by calculating the expected exposures in the studied population. Exposures were calculated using the individual pharmacokinetic parameters and only changing the dose. Doses were assumed to be evenly administered over 4 days in 1-hour infusions and were: 1) 160 mg/m2 for all patients 2) Individualized dosing based on the model, patient weight and serum-creatinine levels 3) Therapeutic drug monitoring with 40 mg/m2 for 1-day and using the measured samples on that day to alter the subsequent doses in order to achieve the target exposure. Panel A represents the full cohort (N=190), Panel B is stratified for children (<20 years, N=72) and panel C is stratified for adults (>20 years, N=118).

9.2.4 Sub-analysis in αβ T cell depleted allograft

A sub-analysis of fludarabine exposure was performed in recipients of a αβT cell depleted allograft. As viral infections are a more frequent complication, we focused on analyzing the impact of fludarabine exposure on ≥ grade III viral infections. This analysis showed that recipients with a fludarabine exposure above the target range have an increased incidence of viral infections ≥ grade III (Figure 7). We hypothesize that this is a consequence of impaired

IR of NK and γδ T cells which associates with overexposure of fludarabine, as these are the main lymphocyte subsets in the first 100 days post αβ T cell depleted allo-SCT [4].

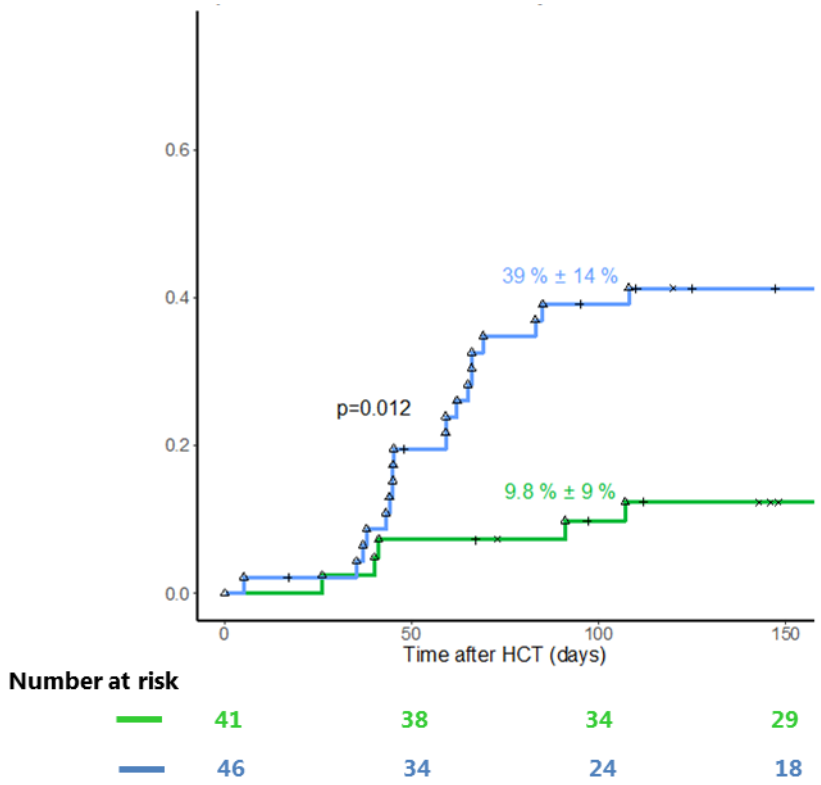


Figure 7: Incidence of viral infections (Y axis) in recipients of αβ T cell depleted allografts with optimal fludarabine levels (green line) or fludarabine levels above optimum (blue line).

9.3 Summary of known and potential risks and benefits

The protocol comprises a different dosing of fludarabine in the experimental arm. All other acts, measurements, follow-up and level of care are therefore similar to off-study patients undergoing allo-SCT. The burden of the therapy is associated with the allo-SCT itself, which is a necessary therapeutic intervention in all subjects. The intended target level of fludarabine in the study group remains in the range of all so far treated patients at the UMCU. We only propose to avoid too high exposure to fludarabine. Possible benefits include a proposed lower incidence of infections, low incidence of GVHD as well as optimized cancer surveillance due to a more balanced immune reconstitution. Possible increased risks for the recipient are associated with too low exposure to fludarabin. The main risk is development graft failures, though the risk of graft failure is in general considered to be low in the intended target population, adults with hematological malignancies receiving myeloablative conditioning[37],. When TDM is used to individualize fludarabin administration, we consider the risk of low fludarabine exposure neglectible (figure 6). When the algorithm is used to dose fludarabin, there might be a risk of low fludarabin exposure. However, based on the

observation that we have not observed any graft failures in the patients treated in the Netherlands according to the current standard arm A (section 4.1.2) which also included patients with low fludarabin exposure, we consider the risk of graft failure when the algorithm is utilized minimal.

Further details on the potential risks of fludarabine may be found in the Summary of Product Characteristics.

9.4 Description and justification of route of administration and dosage

Fludarabin is administered intravenously, as it is always applied in conditioning regimens [24]. The dosage is the subject of this study as outlined in 9.2.

9.5 Dosages, dosage modifications and method of administration

Fludarabine will be administered intravenously for 4 days (days -5 to -2) in 60 minutes and will be prepared by the pharmacy. In arm B dosing of fludarabine will be either individualized based on BSA and renal function according to formula 1# or individualized using therapeutic drug monitoring targeted to a cumulative AUC range of 15-25 mg*h/L (section 8.3).
For TDM blood samples must be drawn on day 1 (at 5 min, 1 hour, 2 hour and 3 hour after end of infusion) and are measured by the laboratory on day 2. Administration of fludarabine on day 2 will take place only after TDM is performed and the advised dose is calculated and communicated to the treating phycisian. Of note: When the first administration of fludarabine takes place on a Saturday, blood samples are drawn on Saturday and Sunday, and the TDM procedure is performed on Monday with a dosing correction before the third administration.In case of dose adjustment of > 25% blood samples are also drawn on day 2 (or 3) (Appendix F).

9.6 Preparation and labelling of Investigational Medicinal Product

Fludarabine will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.
Fludarabine for Injection is supplied as a lyophilized parenteral drug product in single-use vials. Each vial contains 50 mg of fludarabine phosphate.

9.7 Study drug supply

The sponsor (UMCU) won't arrange delivery of fludarabine to trial sites as fludarabine is commercially available to use as part of a conditioning regimen for allo-SCT.

9.8 Drug accountability

The investigator, or a pharmacist or a other appropriate individual who is designated by the investigator, should maintain records of the product's use by each patient. These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable). Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol.

10. METHODS

10.1 Follow-up of primary end points

10.1.1 Severe viral infections (grade II-III according to appendix E)

Severity of infections based on the grading according to the Bone Marrow Transplant Clinical Trials Network (BMT-CTN). See appendix E

10.2 Follow-up of secondary end points

10.2.1 aGVHD

The assessment of eventual occurrence of aGVHD and viral infections is a standard item of each outpatient visit of study and non-study patients. aGVHD is diagnosed and rated on the basis of Gluckberg criteria (Appendix D).

10.2.2 Non relapse mortality / Progression free survival/ Overall survival

As defined by European Society for Blood and Bone Marrow Transplantation (EBMT)[51]. Patients who die before stem cell infusion will be excluded from this analysis, but will be evaluated for (S)AEs and SUSARs.

10.2.3 Graft failure:

- Primary graft failure: failure to achieve an absolute ANC >500/µl at Day +28
- Secondary graft failure: initial neutrophil engraftment followed by a decline in absolute neutrophil count (ANC) <500/µl and unresponsiveness to growth factor therapy. Other causes of pancytopenia are excluded. Unresponsiveness is defined by the treating physician. Date of onset is the first date of ANC < 500/ul after initial engraftment.

10.2.4 Engraftment

For list of definitions of recovery, engraftment, chimerism see also appendix C.

- Neutrophils > 0.5 x 10^9 /L first day of 3 consecutive days
- Platelets > 50 x 10^9 /L : first day of at least 7 days without transfusions
- Chimerism >95% at two consecutive measurements

10.2.5 Levels of ATG, Busulfan, and Fludarabine prior and post SCT

ATG, Busulfan and Fludarabine exposure will be used to perform PK and PD analyses [38-40]. ATG levels will be retrospectively measured post the 2nd and 4th gift of ATG, at day 0 (day of stem cell infusion) and 7 days post infusion in 5 ml of serum.

Blood sampling for TDM Bu and Flu will be done according to the UMC Utrecht protocol "Busulfan administration and blood sampling for TDM" (see appendix F).

10.2.6 Clinical parameters

- Incidence of other than viral infections
- Incidence and grade of chronic GvHD (appendix D)

10.2.7 Immune reconstitution

- True counts of $CD3^+$ T cells, $CD4^+$ and CD8+ subtyping of T cells, CD3-CD16/56+ (NK cells), $\gamma\delta$T-cells
- Extended analysis of immune repertoire, including assessment of $\alpha\beta$, $\gamma\delta$ and NK-cell repertoires[52,53].

10.2.8 Donor cells after αβ T and CD19 B cell depletion

- The total amount of viable $CD34^+$ cells and $CD34^+$ cells/kg recipient will be determined,
- Total αβT-cell number (defined as γδTCR negative and CD3 positive) will be measured after αβ T-/ CD19 B-cell depletion procedure.
- Total CD19+ B cells will be measured after the CD19 αβ T-/ B-cell depletion.
- NK-cell number will be measured after αβ T-/CD19 B-cell depletion procedure.
- γδ T-cell number will be measured after the αβ T-/CD19 B-cell depletion procedure.
- Microbiological contamination will be determined.
- ABO-Rh blood group is determined from the pre harvest donor screening in peripheral blood
- Sex of the donor is known.
- Date and timing of the harvest is known.
- Before the αβ T-/ CD19 B-cell depletion of MUD transplants, part of the stem cell product, (containing unmodified T cells) may be cryopreserved for the later infusion of a potential DLI of 1x10^5 T cells per kg.

10.3 Study procedures

Study periods and enrolment are summarised in this paragraph. Detailed information regarding all assessments and the timing of the study procedures are provided in the schedule of assessments (SoA) (section 10.4)

10.3.1 Screening

Screening evaluations will be performed for all subjects to determine study eligibility.
The screening period begins on the date the subject signs the ICF. Informed consent must be obtained before completion of any non-standard of care study specific procedures.

Procedures that are part of standard of care are not considered study specific procedures and may be performed prior to obtaining consent and used to confirm eligibility.
All screening evaluations must be completed within 28 days of start of conditioning.

10.3.2 Registration and randomization

When a patient has been established as eligible for the study by the local investigator, a registration form must be completed in the Electronic Data Capture (EDC) system. Each patient will be given a unique patient study number (a sequence number by order of enrolment in the trial).
Once the registration form has been controlled and eligibility checked, the patient will be randomized to one of the treatment arms. Arm A: fludarabine classical dosing, Arm B: fludarabine individualized dosing, in a 1:1 ratio using kreatinine clearance (>90 ml/min vs below) as stratification factor.
Random block randomization will be implemented in the randomization module that will be fully integrated within the EDC.
Patient study number and result of randomization will be given immediately by the EDC system and confirmed by email.

10.3.3 Post-treatment follow-up

After completing the treatment period and discharge from the hospital, all subjects will be followed in the Post Treatment Follow-Up period. Counting from Day 0 (= day of HPC(A) infusion) until Day 100.
If a patient prematurely discontinues the study because of any reason before Day 100 all assessments planned for the regular Day 100 visit should be performed at time of discontinuation. This visit will be registered in the EDC system as an early termination visit.

10.3.4 Long term follow-up

All enrolled subjects will be followed in the long term follow-up period until relapse/progression. Subjects will begin the long term follow-up period after they have completed the EOT visit.
After relapse/progression, subjects will be followed for overall survival and subsequent anticancer therapies.

10.4 Schedule of Assessments

Study period or visit	**screening**	**Conditioning**		**Infusion HPC (A)**	**Post-treatment follow-up**			**Long term follow-up**					**OS**
Day/month	-28 days of start conditioning	Day -12 to -9	Day -5 to -2	Day 0	Month 1	Month 2	**day 100 / EOT**	Month 4	Month 5	Month 6	Month 9	Month 12	Every 6 months
Window					± 5 days	± 5 days	**± 3 days**	± 7 days	± 7 days	± 7 days	± 2 weeks	± 3 weeks	± 3 weeks
General activities													
Informed consent	x												
Demographics, medical history	x												
Pre-SCT evaluations[i]	x												
Physical examination[ii] / WHO score [iii]	x				x	x	**x**	x	x	x			
Adverse events [iv]		x	x	x	x	x	**x**						
Disease assessment [v]	x						**x**			x	x	x	
GVHD assessment [vi]					x	x	**x**	x	x	x	x	x	
Survival status/subsequent therapies													x
ATG administration		x											
Fludarabine+ Busulfan administration			x										
$\alpha\beta$TCR/CD19 depleted graft				x									
Laboratory Evaluations													
Haematology [vii]	x	x	x	x	x	x	**x**	x	x	x	x	x	
Serum chemistry [viii]	x	x	x	x	x	x	**x**	x	x	x	x	x	
PCR CMV, EBV [ix]					x	x	**x**	x	x	x	x	x	
PB Chimerism (T and non-T) [x]	x			x	x	x	**(x)**	(x)	(x)	(x)	(x)	(x)	
Immune monitoring[xi]	x				x	x	**x**	x	x	x	x	x	
Sampling for biological studies[xii]	x				x	x	**x**	x	x	x	x	x	
ATG levels[xiii]		x		x									
Fludarabine + Busulfan levels [xiv]			x										

[i] Patients will be evaluated before transplantation according to local protocol and international guidelines (JACIE), which includes blood and bone-marrow sampling

[ii] Including weight, signs of toxicity, infection

[iii] According to WHO classification, (see appendix A)

[iv] According to protocol section 11

[v] Disease assessment per local guidelines as applicable per haematological malignancy. Including BM morphology, immunophenotyping and MRD if applicable. Relapse/proression of disease to be confirmed according to international criteria per haematological malignancy.

[vi] GVHD assessment: According to appendix D

[vii] Haematology: Complete Blood Count with differential three times a week from day 0 until ANC>0.5 x 109/l for 2 consecutive measurements and subsequently on each visit in the outpatient clinic.

[viii] Chemistry: electrolytes, albumin, glucose, creatinine, bilirubin, ALT/AST, alkaline phosphatase at least once weekly during admission and subsequently at each outpatient clinic visit.

[ix] During hospitalization 1x/week, thereafter every outpatient clinic visit for a year. This is part of standard care and will be performed according to the local guidelines

[x] Chimerism: monthly starting from day 30 until 6 month after allo-SCT. Stop if chimerism is twice >95% at month 2. Prolonged measurement of chimerism if not >95% after 2 months, then monthly until twice >95%.

[xi] Total (true) counts of B-, T-, CD4, CD8, NK and γδT-cells (vδ2+ and vδ2- subsets). From Leucocytes >0.4 x 109 /L. During clinical admission every two weeks, thereafter, every month

[xii] At all time points, blood samples for exploratory endpoints must be obtained (45ml peripheral blood). Bone marrow sampling is performed at month 3, 6, and 12, a sample for exploratory endpoints should also be obtained (9mL). *For lymphoma only applicable if bone marrow was involved at baseline.*

[xiii] ATG levels will be retrospectively measured post the 2nd and 4th dose of ATG, at day 0 (day of stem cell infusion) and 7 days post infusion in 5 ml of serum

[xiv] Fludarabine and busulfan levels: blood sampling (2mL PB) on Day 1 at 5 minutes, 1, 2 and 3 hours after administration (appendix F).

10.5 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 can decide to withdraw a subject from the study for urgent medical reasons.

10.6 Replacement of individual subjects after withdrawal

Individual subjects will be replaced after withdrawal if the transplantation procedure is cancelled.

10.7 Other reasons for going off study

- Death
- Major protocol violation
- Graft failure
- Relapse/progression

Patients who are withdrawn from the study treatment for other reasons than death will be followed only for overall survival. Data obtained from patients who are withdrawn from the study treatment for other reasons than withdrawal of their consent will be used for statistical analysis.

10.8 Premature termination/alteration of the study

The sponsor (UMCU) may decide to terminate the study prematurely based on the following criteria:

- There is evidence of an unacceptable risk for study patients (i.e. safety issue)
- The DSMB recommends ending the trial based on viable arguments (e.g. insufficient enrollment of patients). Statistical analyses for all decisions are available under section 12.

11. SAFETY REPORTING

11.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor (UMCU) will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited ethics committee (EC) 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 accredited EC. The investigator will take care that all subjects are kept informed.

11.2 Adverse events

11.2.1 Definition AE

Adverse events (AE) are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the study treatment. All adverse events reported spontaneously by the subject or observed by the investigator or its staff will be recorded, excluding exceptions defined in section 11.2.2.

11.2.2 Reporting AEs

Adverse events will be reported from the start of the conditioning until 100 days following stem cell infusion. Adverse events have to be reported on the Adverse Events case report form (CRF). Adverse events will be scored according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.03.

All Adverse Events have to be reported, with the exception of:

- A pre-existing condition that does not increase in severity; the pre-existing condition should be reported on the baseline concomitant diseases CRF
- AEs of CTCAE < grade III
- Mucositis CTCAE < grade IV
- All hematological toxicities (unless SAE), alopecia, nausea and vomiting
- AEs directly contributable to ATG infusion (e.g. hypotension, fluid overload); unless SAE.
- AEs directly contributable to stem cell infusion / infusion blood products resolving < 24 hours (e.g. hypotension, fever); unless SAE.
- Neutropenic fever without a clinical substrate, responding to i.v. antibiotics < 72 hours.
- GvHD, to be reported on specific GvHD CRF
- Viral infections (CMV/EBV/BK cystitis), to be reported on infection CRF.

11.3 Serious adverse events (SAEs)

11.3.1 Definition SAE

A serious adverse event 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 in-patients' hospitalization;
- results in persistent or significant disability or incapacity;

- is a congenital anomaly or birth defect;
- 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.

An elective hospital admission will not be considered as a serious adverse event.

11.3.2 Reporting SAEs

SAEs must be reported to the research team of the Hematology department of the UMCU **within 24 hours** by email hemat-research@umcutrecht.nl after the event was known to the investigator, using the SAE report form provided. This initial report should contain a minimum amount of information regarding the event, associated treatment and patient identification, as described in the detail in the instructions for the SAE report form. Complete detailed information should be provided in a follow-up report within a further 2 business days, if necessary.

The sponsor (UMCU) will report the SAEs through the web portal ToetsingOnline to the accredited EC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 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 serious adverse events.

11.3.3 Causality assessment of SAEs

The investigator will decide whether the SAE is related to the study treatment.The assessment of causality is made by the investigator using the following:

RELATIONSHIP	DESCRIPTION
UNRELATED	There is no evidence of any causal relationship
UNLIKELY	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the patient's clinical condition, other concomitant treatments).
POSSIBLE	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the patient's clinical condition, other concomitant treatments).
PROBABLE	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
DEFINITELY	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
NOT ASSESSABLE	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

11.4 Suspected unexpected serious adverse reaction (SUSAR)

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

1. the event must be serious (see chapter 11.3.1.);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. 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 as recorded in:
    a. the SPC for an authorised medicinal product;
    b. Investigator's Brochure for an unauthorised medicinal product.

SUSARs must be reported to the research team of the Hematology department of the UMCU within 24 hours by email hemat-research@umchtrecht.nl after the event was known to the investigator, using the SAE report form provided

The sponsor (UMCU) will ensure the reporting of any SUSARs to the Ethics Committees (EC), the Competent Authorities (CA) and the investigators in compliance with applicable laws and regulations.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

11.5 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor (UMCU) will submit, once a year throughout the clinical trial, a safety report to the accredited Ethics Committees, competent authority, and competent authorities of the concerned Member States.
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, per study;
- 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 medicine under investigation.

11.6 Follow-up of adverse events

All adverse events 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.
Follow-up reports on SAEs will be added to the reported SAEs on *ToetsingOnline*.

11.7 Data Safety and Monitoring Board

The Data and Safety Monitoring Board will advise the Principal Investigator and the Co-investigator(s) about the continuation of the study. The DSMB will evaluate the general progress and the feasibility of the study, the quality and completeness of the data, side effects and safety.

The DSMB consists of at least 3 members, among whom (at least) one statistician and minimally two physicians. The members of the DSMB are invited on personal title on the basis of their expert knowledge of the disease involved or the research methodology.
Members of the DSMB will have ample experience with randomized clinical trials.
The members of the DSMB will not be involved in the study or work in a hospital department participating in the study. The members will not have a conflict of interest due to ties with a company involved in the study.

The DSMB reports their written recommendations to the Principal Investigator and the Co-investigator(s). The DSMB recommendations are not binding. Should the sponsor (UMCU)decide not to fully implement the advice of the DSMB, the sponsor will send the advice to the reviewing Ethics Committees, including a note to substantiate why (part of) the advice of the DSMB will not be followed.
The DSMB will receive at least the following reports from the trial statistician for review:

- Annual safety data listing the incidence of (serious) adverse events and (serious) adverse reactions
- Annual progress data listing the number of enrolled patients and the status of data collection
- Annual report on safety outcomes

12. STATISTICAL CONSIDERATIONS

12.1 Patient numbers and power considerations

The aim of the randomization is to evaluate whether addition of targeted fludarabine results in a reduction of the incidence of severe viral infections. For the primary endpoint, patients will be considered a success if they did not develop serious infections within 100 days post-transplant while being alive, relapse-free. Based on the retrospective data, we hypothesize that 40 % of the subjects treating according to arm A will experience serious infections. To detect a 25% reduction of failures with arm B with a power of 80%, each arm should consist of 42 patients evaluable at day 100. This is based on the assumption that 10% of patients in each arm will not contribute to the primary analysis due to death or relapse. This leads to an assumed proportion of failures of 40/90=0.444444 in arm A and 15/90=0.1666667 in arm B (2-sided significance level of 0.05, Z test with pooled variance, power=81%). To compensate for non-evaluability due to death, relapse or other reasons, 15% extra patients per arm will be included, leading to a total sample size of 2 times 49, is 98 patients.

12.2 Safety

With respect to safety, a yearly report as described in 11.5 and 11.7 will be submitted to the EC and DSMB. In case 20 patients reach the 100 days follow-up before 1 year, the DSMB will be informed earlier. The yearly report will contain NRM, OS and relapse rates. In addition we will report the reportes (S)AEs and SUSARs. All patients will be considered in the safety analysis. Patients will be closely followed for unexpected toxicities and, if any serious side effects are observed, the investigators will reevaluate the appropriate course for the study. At the conclusion of the study, all unexpected toxicities will be summarized and reported. If the DSMB recommends to stop the study based on the safety outcomes, the sponsor (UMCU)

will follow the advice of the DSMB or discuss with the EC requirements for continuation of the study.

12.3 Interim analysis

No interim analysis will be performed. Yearly reports will be submitted to the DSMB.

12.4 Statistical analysis plan

All analyses will be done in accordance with the intention-to-treat principle in all patients who have initiated the study treatment, when the last patient is 100 days post transplantation or has gone 'off-protocol'.

12.4.1 Primary endpoint

The primary endpoint is the probability of serious viral infections up to 100 days after transplantation for patients alive relapse-free at 100 days. This endpoint will be analyzed in a poisson regression model including treatment arm and the stratification factors used in the randomization.

12.4.2 Secondary endpoints

All time-to-event endpoints are measured from the date of stem cell infusion. Cumulative incidence of aGVHD grade II-IV is to the time to aGVHD II-IV at day 100 with relapse and death as competing events. A 95% confidence interval will be constructed. Overall survival (OS) is defined as the probability to be alive. EFS is defined as the time interval of being alive without relapse/disease progression or graft failure. Relapse incidence (RI) is defined as probability of relapse/progression, with death as a competing event. Non-relapse mortality (NRM) is defined as probability of death without relapse or progression. Time to engraftment will be analysed as a competing risks outcome with competing event death without engraftment.

OS and EFS will be estimated by the Kaplan-Meier product method. Competing risks outcomes will be estimated by cumulative incidence curves and will also utilize models based on cause-specific hazard models. 95% confidence intervals will be constructed.

12.4.3 ATG and fludarabine exposure

ATG and fludarabine exposure will be measured as cumulative exposure, which will be correlated to immune reconstitution and clinical outcome.

12.4.4 Toxicity analysis

The analysis of treatment toxicity will be done primarily by tabulation of the incidence of adverse events and infections with CTCAE grade 3 or 4, excluding AEs as outlined in 11.2.2.

13. ETHICAL CONSIDERATIONS

13.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki 1964 (last amended by the 64th WMA General Assembly in October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO) and Good Clinical Practice. The accredited EC will approve the study protocol and any substantial amendments.

13.2 Recruitment and consent

After referral for allo-SCT, for each patient the best treatment and donor will be discussed in a SCT meeting. For patients eligible to the inclusion criteria of this study, this study will be considered as one of the options. If a benefit is argument in favor of this study and this αβTCR/CD19 depleted allo-SCT protocol is considered to be the best option, the responsible SCT doctor will discuss the treatment proposal with the patient. This is always done during the second visit.

Written informed consent of patients is required before enrolment in the trial and before any study related procedure takes place.

The investigator will follow ICH-GCP and other applicable regulations in informing the patient and obtaining consent. The investigator should take into consideration if the patient is capable of giving informed consent. Before informed consent may be obtained, the investigator should provide the patient ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the patient.

There is no set time limit for the patient to make a decision. The investigator should inform each patient if there is a specific reason why he/she must decide within a limited time frame, for example if patients condition necessitates start of treatment or if the trial is scheduled to close for enrolment.

The content of the patient information letter, informed consent form and any other written information to be provided to patients will be in compliance with ICH-GCP and other applicable regulations and should be approved by the Ethics Committee in advance of use. The patient information letter, informed consent form and any other written information to be provided to patients will be revised whenever important new information becomes available

that may be relevant to the patient's consent. Any revised informed consent form and written information should be approved by the Ethics Committee in advance of use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient's willingness to continue participation in the trial. The communication of this information should be documented.

13.3 Benefits and risks assessment

13.3.1 Burden

The protocol comprises a different dosing of fludarabine in the experimental arm. In addition, extra blood (9 times 45 ml) and bone marrow (4 times 5 ml) will be sampled for exploratory endpoints. Sampling will take place at moments blood or bone marrow sampling was scheduled for routine clinical care. The intervals of sampling are > 1 month. The average blood volume in humans is 4.5 – 5.6 liter. We don't expect that donation of maximum 1% of the total blood volume will lead to cytopenias and/or an increase of bloodtransfusions. Bone marrow sampling for exploratory endpoints will always be executed after bone marrow has been sampled for clinical monitoring.

All other acts, measurements, follow-up and level of care are similar to off-study patients undergoing allo-SCT. The burden of the therapy is associated with the allo-SCT itself, which is a necessary therapeutic intervention in all subjects.

13.3.2 Risks

Possible increased risks for the recipient are graft failures, though not observed so far in all cohorts with the intended dose levels. The intended target level of fludarabine remains in the range of all so far treated patients at the UMCU. We only propose to avoid too high exposure to fludarabine.

13.3.3 Benefits

Possible benefits include a proposed lower incidence of infections as well as optimized cancer surveillance due to a more balanced immune reconstitution.

13.4 Compensation for injury

The sponsor (UMCU) has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 650.000,-- (i.e. six hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 5.000.000,-- (i.e. five million Euro) for death or injury for all subjects who participate in the Research;
3. € 7.500.000,-- (i.e. seven million five hundred thousand Euro) for the total damage incurred by the organization for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

14. DATA COLLECTION AND QUALITY ASSURANCE

14.1 Electronic Data Capture (EDC)

Data will be collected on electronic Case Report Forms (eCRF) in the EDC system to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected in the EDC system are derived from the protocol and will include at least:

- inclusion and exclusion criteria;
- baseline status of patient including medical history and stage of disease;
- timing and dosage of protocol treatment;
- (severe) adverse events;
- parameters for response evaluation;
- any other parameters necessary to evaluate the study endpoints;
- survival status of patient;
- reason for end of protocol treatment.

The eCRFs will be completed on site by the local investigator or an authorized staff member. Each eCRF must be dated and signed by the local investigator upon completion. All eCRF entries must be based on source documents. The eCRF and instructions for completing the eCRF will be provided by the UMC Utrecht Hematology data-management.

14.2 Monitoring

Independent monitors of the Julius Clinical BV will perform on-site monitoring visits to verify that trial conduct at the site is in compliance with ICH-GCP and the applicable regulatory requirements, as depicted in the 'monitoring plan'.
According to the NFU risk classification, we consider the risk as 'moderate'. As stated before, the greatest hazard for the patient is determined by the allo-SCT as such. Additional side

effects may be an increased risk of graft failures or GVHD, though not observed so far in all cohorts with the intended dose levels. The intended target level of fludarabine remains in the range of all so far treated patients at the UMCU. We only propose to avoid too high exposure to fludarabine. Blood and bone marrow sampling for biological studies are not expected to induce any side effects.

15. ADMINISTRATIVE ASPECTS AND PUBLICATION

15.1 Handling and storage of data and documents

15.1.1 Patient confidentiality

Each patient is assigned a unique patient study number at registration. In trial documents the patient's identity is coded by patient study number as assigned.
The local investigator will keep a subject enrolment and identification log that contains the key to the code, i.e. a record of the personal identification data linked to each patient study number. This record is filed at the investigational site and should only be accessed by the investigator and the supporting site staff, and by representatives of the sponsor (UMCU) or a regulatory agency for the purpose of monitoring visits or audits and inspections.

15.1.2 Filing of essential documents

Essential Documents are those documents that permit evaluation of the conduct of a trial and the quality of the data produced. The essential documents may be subject to, and should be available for, audit by the sponsor's auditor and inspection by the regulatory authority(ies).
The investigator should file all essential documents relevant to the conduct of the trial on site.
The sponsor (UMCU) will file all essential documents relevant to the overall conduct of the trial. Essential documents should be filed in such a manner that they are protected from accidental loss and can be easily retrieved for review.

15.1.3 Record retention

Essential documents should be retained for 15 years after the end of the trial. They should be destroyed after this time.
Source documents (i.e. medical records) of patients should be retained for at least 15 years after the end of the trial. Record retention and destruction after this time is subject to the site's guidelines regarding medical records.

15.1.4 Storage of samples

Biological samples should only be stored for the purpose of additional research if the patient has given consent. If no informed consent was obtained, samples should be destroyed after the patient has completed all protocol treatment and procedures.
Storage of biological samples on site is subject to the site's guidelines; samples are stripped from any identifying information and labelled with a code (trial name or number and patient study number as assigned at enrolment).

15.1.5 Disclosure of an extract of the data with Miltenyi Biotec

Miltenyi Biotec has developed the Clinimacs ® TCRα/β Product line for graft engineering. For post marketing research, Miltenyi will receive an extraction of the data obtained in this study from subjects which have given consent. Applicable Data Privacy Laws will be followed. The dataset will contain data with regards to clinical outcome, immuno reconstitution and graft composition. Data cannot be linked to participants of this study.

15.2 Amendments

A 'substantial amendment' is defined as an amendment to the terms of the EC 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 EC and to the competent authority.

Non-substantial amendments will not be notified to the accredited EC and the competent authority, but will be recorded and filed by the sponsor (UMCU).

15.3 Annual progress report

The sponsor (UMCU) will submit a summary of the progress of the trial to the accredited EC 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.

15.4 End of study report

The sponsor (UMCU) will notify the accredited EC and the competent authority 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 EC 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 EC and the competent authority 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 accredited EC and the Competent Authority.

15.5 Public disclosure and publication policy

The results of this trial will be disclosed unreservedly according to the rules of the CCMO statement on publication policy (www.ccmo.nl). If it cannot be published in peer reviewed journals, we will disclose the results to an international trial register (het Nederlands trial register: www.trialregister.nl and the EBMT database. The involved physicians will receive the draft of the manuscript to allow them to give their input. The order of the authors will be discussed in function of the amount of work given on each manuscript and the number of cases included.

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17. Appendices

APPENDIX A. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

2 Some bed time, but to be in bed less than 50% of normal daytime

3 Needs to be in bed more than 50% of normal daytime

4 Unable to get out of bed

APPENDIX B. NYHA* scoring list

Grade 1 No breathlessness

Grade 2 Breathlessness on severe exertion

Grade 3 Breathlessness on mild exertion

Grade 4 Breathlessness at rest

The *New York Heart Association functional and therapeutic classification applied to dyspnoea

APPENDIX C. Definitions of recovery, engraftment and chimerism

Neutrophil recovery: First of 2 consecutive days with neutrophils ≥ 0.5 x 10^9/l

Platelet recovery: First of 2 consecutive days with platelets ≥ 20 x 10^9/l without platelet support for 7 days

Engraftment: Neutrophil recovery in association with donor hematopoiesis > 10% in bone marrow

Primary graft failure: Cytopenia and marrow hypoplasia after 60 days with donor hematopoiesis < 10%

Secondary graft failure: Complete loss of donor hematopoiesis after initial engraftment.

Complete chimerism: >95% donor hematopoiesis, < 5% recipient hematopoiesis in bone marrow

Mixed chimerism: 10-95% donor hematopoiesis and >5% recipient hematopoiesis in bone marrow

Autologous reconstitution: >95% recipient hematopoiesis, < 5% donor hematopoiesis in bone marrow

APPENDIX D. Grading of GVHD

Acute GVHD

For staging and grading the Glucksberg classification updated according to Przepiorka et al will be used[41].

Stage	Skin	Liver	Intestinal Tract	
	Rash (% body surface)**	Total bilirubin (μmol/L)	Diarrhea (ml/day)	
1	< 25	34-50	500-1000 or persistent nausea without diarrhea *	280-555 ml/m^2
2	25-50	50-102	1000-1500	556-833 ml/m^2
3	> 50	102-255	> 1500	> 833 ml/m^2
4	Generalized erythroderma with bullous formation	> 255	3. + severe abdominal pain / ileus	

*persistent nausea with histologic evidence of GVHD in the stomach or duodenum

** For a body surface area < 1.73 m^2 calculation per m^2

Grade	
I	Skin: stage 1-2 and Liver: stage 0 and Gut: stage 0
II	Skin: stage 3 or Liver: stage 1 or Gut: stage 1
III	Liver: stage 2-3 or Gut: stage 2-4
IV	Skin or Liver: stage 4

Chronic GVHD

NIH consensus criteria for GVHD severity

A scoring system for chronic GVHD severity was created at a consensus conference supported by the National Institutes of Health (NIH) in 2005 and revised in 2014[42].

The NIH GVHD scoring system includes information on the number of organs or sites involved and the severity within each affected organ (eg, skin, mouth, eyes, gastrointestinal tract, liver, lungs, joints/fascia, and genital tract)[42]. Organ specific severity is scored from 0 to 3 with higher scores reflecting more severe disease. Based upon this information, the overall severity is scored as mild, moderate, or severe:

- **Mild** – Involves two or fewer organs/sites with no clinically significant functional impairment
- **Moderate** – Involves three or more organs/sites with no clinically significant functional impairment or at least one organ/site with clinically significant functional impairment, but no major disability
- **Severe** – Major disability caused by chronic GVHD

Grading of chronic GvHD as described by Jagasia et al.[42] should be performed as described below.

	Score 0	Score 1	Score 2	Score 3
Performance score: ☐ KPS ECOG LPS	☐ Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	☐ Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80 to 90%)	☐ Symptomatic, ambulatory, capable of self-care, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60 to 70%)	☐ Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3 to 4, KPS or LPS <60%)
Skin* **Score % BSA** ☐ *GVHD features to be scored by BSA:* ***Check all that apply:*** ☐ Maculopapular rash/ erythema ☐ Lichen planus-like features ☐ Sclerotic features ☐ Papulosquamous lesions or ichthyosis ☐ Keratosis pilaris-like GVHD	☐ No BSA involved	☐ 1 to 18% BSA	☐ 19 to 50% BSA	☐ >50% BSA
Skin features **Score:**	☐ No sclerotic features		☐ Superficial sclerotic features "not hidebound" (able to pinch)	***Check all that apply:*** ☐ Deep sclerotic features ☐ "Hidebound" (unable to pinch) ☐ Impaired mobility ☐ Ulceration
Other skin GVHD features (NOT scored by BSA) ***Check all that apply:*** ☐ Hyperpigmentation ☐ Hypopigmentation ☐ Poikiloderma ☐ Severe or generalized pruritus ☐ Hair involvement ☐ Nail involvement ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
Mouth *Lichen planus-like features present:* ☐ Yes ☐ No	☐ No symptoms	☐ Mild symptoms **with** disease signs but not limiting oral intake significantly	☐ Moderate symptoms with disease signs **with** partial limitation of oral intake	☐ Severe symptoms with disease signs on examination **with** major limitation of oral intake
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

	Score 0	Score 1	Score 2	Score 3
Eyes *Keratoconjunctivitis sicca (KCS) confirmed by ophthalmologist* ☐ Yes ☐ No ☐ Not examined	☐ No symptoms	☐ Mild dry eye symptoms not affecting ADL (requirement of lubricant eye drops ≤3 x per day)	☐ Moderate dry eye symptoms partially affecting ADL (requiring lubricant eye drops >3 x per day or punctal plugs), **WITHOUT** new vision impairment due to KCS	☐ Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** unable to work because of ocular symptoms **OR** loss of vision due to KCS
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
GI tract ***Check all that apply:*** ☐ Esophageal web/proximal stricture or ring ☐ Dysphagia ☐ Anorexia ☐ Nausea ☐ Vomiting ☐ Diarrhea ☐ Weight loss ≥5%¶ ☐ Failure to thrive	☐ No symptoms	☐ Symptoms without significant weight loss¶ (<5%)	☐ Symptoms associated with mild to moderate weight loss¶ (5 to 15%) **OR** moderate diarrhea without significant interference with daily living	☐ Symptoms associated with significant weight loss¶ >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation **OR** severe diarrhea with significant interference with daily living
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
Liver	☐ Normal total bilirubin and ALT or AP <3 x ULN	☐ Normal total bilirubin with ALT ≥3 to 5 x ULN or AP ≥3 x ULN	☐ Elevated total bilirubin but ≤3 mg/dL or ALT >5 ULN	☐ Elevated total bilirubin >3 mg/dL
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				
LungsΔ **Symptom score:**	☐ No symptoms	☐ Mild symptoms (shortness of breath after climbing one flight of steps)	☐ Moderate symptoms (shortness of breath after walking on flat ground)	☐ Severe symptoms (shortness of breath at rest; requiring O_2)
Lung score: % FEV1 ☐	☐ FEV1 ≥80%	☐ FEV1 60 to 79%	☐ FEV1 40 to 59%	☐ FEV1 ≤39%
Pulmonary function tests ☐ Not performed ☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____				

	Score 0	Score 1	Score 2	Score 3
Joints and fascia **P-ROM score** *(see below)* Shoulder (1 to 7):_____ Elbow (1 to 7):_____ Wrist/finger (1 to 7):_____ Ankle (1 to 4):_____	☐ No symptoms	☐ Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	☐ Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, decrease ROM **AND** mild to moderate limitation of ADL	☐ Contractures **WITH** significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self, etc)

☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____

Genital tract ☐ Not examined ***Currently sexually active:*** ☐ Yes ☐ No	☐ No signs	☐ Mild signs◇ and females with or without discomfort on exam	☐ Moderate signs◇ and may have symptoms with discomfort on exam	☐ Severe signs◇ with or without symptoms

Check all signs that apply:

☐ Lichen planus-like features
☐ Lichen sclerosis-like features
☐ Vaginal scarring (female)
☐ Clitoral/labial agglutination (female)
☐ Labial resorption (female)
☐ Erosions
☐ Fissures
☐ Ulcers
☐ Phimosis (male)
☐ Urethral meatus scarring/stenosis (male)

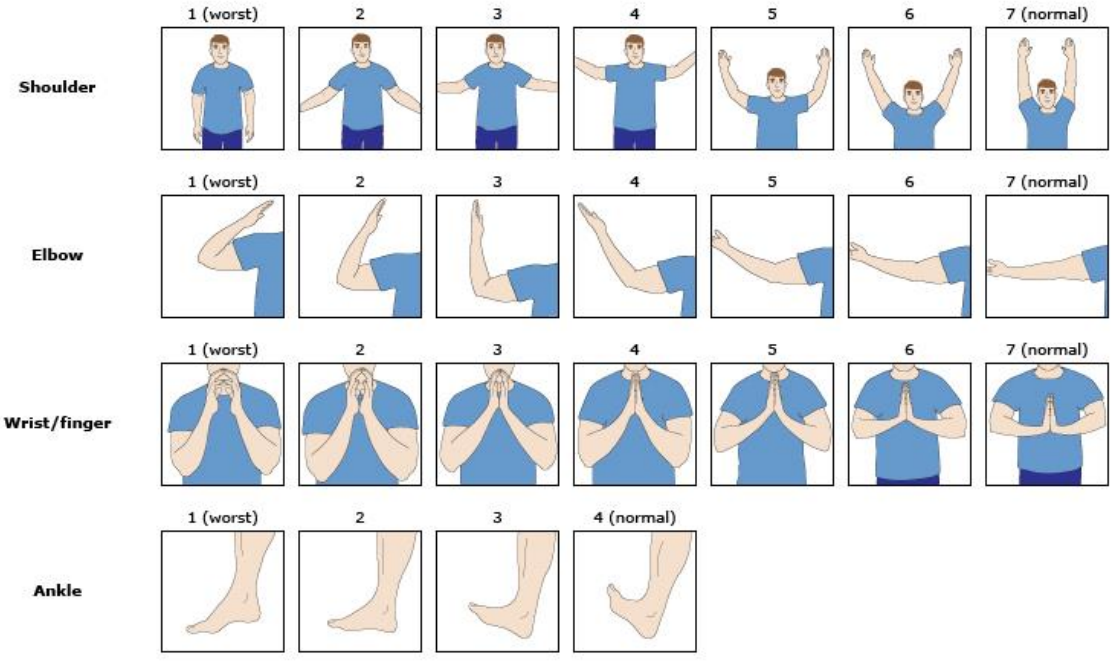
☐ *Abnormality present but explained entirely by non-GVHD documented cause (specify):*_____
☐ *Abnormality present but **NOT** thought to represent GVHD (specify):*_____
☐ *Abnormality thought to represent GVHD **PLUS** other causes (specify):*_____

Other indicators, clinical features or complications related to chronic GVHD (check all that apply and assign a score to severity (0 to 3) based on functional impact where applicable, none = 0, mild = 1, moderate = 2, severe = 3)

☐ Ascites (serositis)_____
☐ Pericardial effusion_____
☐ Pleural effusion(s)_____
☐ Nephrotic syndrome_____
☐ Myasthenia gravis_____
☐ Peripheral neuropathy_____
☐ Polymyositis_____
☐ Weight loss >5%¶ without GI symptoms_____
☐ Eosinophilia >500/microL_____
☐ Platelets <100,000/microL_____
☐ Others (specify):_____

Overall GVHD severity *(opinion of the evaluator)* ☐ **No GVHD** ☐ **Mild** ☐ **Moderate** ☐ **Severe**

Photographic range of motion (P-ROM)



GVHD: graft-versus-host disease; KPS: Karnofsky Performance Status; ECOG: Eastern Cooperative Oncology Group; LPS: Lansky Performance Status; BSA: body surface area; ADL: activities of daily living; LFTs: liver function tests; AP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ULN: upper limit of normal.

* Skin scoring should use both percentage of BSA involved by disease signs and the cutaneous features scales. When a discrepancy exists between the percentage of total body surface (BSA) score and the skin feature score,

OR if superficial sclerotic features are present (score 2), but there is impaired mobility or ulceration (score 3), the higher level should be used for the final skin scoring.

¶ Weight loss within three months.

Δ Lung scoring should be performed using both the symptoms and FEV1 scores whenever possible. FEV1 should be used in the final lung scoring where there is discrepancy between symptoms and FEV1 scores.

◊ To be completed by specialist or trained medical providers

APPENDIX E. Severity of viral infections

Viral infection severity grading

	Grade 1	**Grade 2**	**Grade 3**
HSV	Mucous HSV		Severe HSV (e.g. organ involvement, encephalitis, keratitis)
VZV	Dermatol zoster	VZV infection with 3 or more dermatomes	Severe VZV (e.g. coagulopathy, encephalitis)
CMV*	Asymptomatic CMV viremia, with a > 66% decline of the baseline value decline in viral load after 2 weeks of oral therapy with valganciclovir, measured in the week after cessation of therapy	Clinically active CMV infection (e.g. symptoms, cytopenias) or asymptomatic CMV viremia, with less than 66% decline of the baseline value decline in viral load after 2 weeks of oral therapy, , measured in the week after cessation of therapy **OR** asymptomatic CMV viremia requiring foscarvir	CMV end-organ involvement (pneumonitis, enteritis, retinitis)
EBV^	EBV reactivation not treated with rituximab	EBV reactivation requiring rituximab	EBV PTLD
AdV	Adenoviral conjunctivitis, asymptomatic viruria, asymptomatic stool shedding and viremia not requiring treatment	Adenoviral upper respiratory infection, viremia **OR** symptomatic viruria requiring treatment	Adenovirus with end-organ involvement (except conjunctivitis and upper respiratory tract)
HHV 6	Asymptomatic HHV-6 load	Clinically active HHV- 6 infection (e.g. symptoms, cytopenias) **OR** HHV-6 viremia requiring therapy	
BK	BK viremia or viruria with cystitis not requiring intervention	BK viremia or viruria with clinical consequence requiring (prolonged) admission to the hospital **AND/OR** urological intervention	
Enterocolitis	Enterocolitis with virus present in the stool, not requiring intervention	Enterocolitis with virus present in the stool, requiring (prolonged) admission to the hospital for supportive care	
Respiratory	Respiratory tract viral	Respiratory tract viral	Respiratory tract viral

tract infection	infection with documented virus not requiring intervention or admission to the hospital	infection with documented virus requiring (prolonged) admission to the hospital **OR** viral respiratory infection complicated by a documented bacterial superinfection	infection requiring mechanical ventilation
Viral infections not specified above	Documented viral infection other as viral infections defined above not requiring intervention or admission to the hospital	Documented viral infection other as viral infections defined above requiring intervention or admission to the hospital	

*Definition CMV reactivation: viral load in blood > 250 IU/ml.

^Definition asymptomatic EBV reactivation: EBV detectable in blood not requiring treatment. EBV reactivation requiring treatment: EBV load > 1000 IU/ml OR detectable EBV load with clinical symptoms OR persistent elevated EBV load < 1000 IU/ml considered significant by the treating physician

Severity of infections based on the grading according to the Bone Marrow Transplant Clinical Trials Network (BMT-CTN). https://web.emmes.com/study/bmt2/public/Definition/Severity%20Grading_Recurrence%20Interval.pdf).

APPENDIX F. Blood sampling for TDM Bu and Flu

Blood sampling for TDM for both Bu and Flu will be done according to the UMC Utrecht protocol, available at the UMCU website: https://www.umcutrecht.nl/nl/Ziekenhuis/Professionals/Diagnostiek-aanvragen/Farmalab. Choose Busulfan or Fludarabin where applicable.