Clinical Study Protocol

Oral complications in patients treated with hematopoietic stem cell transplantation

ORA-STEM study

Principal Investigators: Dr. M.D. Hazenberg
Department of Hematology, AMC
Amsterdam, The Netherlands
Email: m.d.hazenberg@amc.uva.nl

Dr. J.E. Raber-Durlacher
Department of Oral and Maxillofacial Surgery, AMC
Amsterdam, The Netherlands
Email: j.e.raberdurlacher@amc.uva.nl

Study coordinator: Dr. A.M.G.A. van Bruchem-Laheij
Department of Medical Dental Interaction, ACTA
Department of Oral and Maxillofacial Surgery, AMC
Email: a.laheij@acta.nl

C**o-Investigators** Drs. T.M. Haverman
Department of Medical Dental Interaction, ACTA
Amsterdam, The Netherlands
Email: thijs.haverman@acta.nl

Drs. S. Al-Ansari
Department of Medical Dental Interaction, ACTA
Department of Oral and Maxillofacial Surgery, AMC
Amsterdam, The Netherlands
Email: research.alansari@gmail.com

Prof. dr. F.R. Rozema

Department of Medical Dental Interaction, ACTA

Amsterdam, The Netherlands

Email: fred.rozema@acta.nl

Prof. dr. N.M.A. Blijlevens

Department of Hematology, RUMC

Nijmegen, The Netherlands

Email: n.blijlevens@hemat.umcn.nl

Prof. dr. M.C. D.N.J.M. Huysmans

Department of Cariology and Endodontology, RUMC

Nijmegen, The Netherlands

Email: m.c.d.n.j.m.huysmans@dent.umcn.nl

Dr. J.J. de Soet

Department of Experimental Preventive Dentistry Cariology

Academic Centre for Dentistry Amsterdam

Amsterdam, The Netherlands

Email: j.d.soet@acta.nl

Dr. A. Mank

Department of Hematology, AMC

Amsterdam, The Netherlands

Email: a.mank@ amc.uva.nl

Dr. C. M.J. Potting

Department of Hematology, RUMC

Nijmegen, The Netherlands

Email: Carin.Potting@radboudumc.nl

Prof. Dr. J. de Lange

Department of Oral and Maxillofacial Surgery, AMC

Amsterdam, The Netherlands

Email: j.de lange@amc.uva.nl

Drs. J. Bos-den Braber

Department of Cariology and Endodontology, RUMC

Nijmegen, The Netherlands

Jacolien.Bos-denBraber@radboudumc.nl.

Prof. Dr M.J. Kersten

Department of Hematology, AMC

Amsterdam, The Netherlands

Email: m.j.kersten@amc.uva.nl

Principal Investigators

International:

Prof. dr. M.T Brennan

Department of Oral Medicine

Carolinas Medical Center

Charlotte, North Carolina USA

Email: Mike.Brennan@carolinashealthcare.org

Prof. dr. I. von Bültzingslöwen

Department of Oral Medicine

University Hospital University of Gothenburg, Sweden

Email: ingervonb@hotmail.com

Trial statistician:

Prof. dr. A.H. Zwinderman

Academic Medical Centre, University of Amsterdam

Department of Clinical Epidemiology and Bio-Statistics

Email: a.h.zwinderman@amc.uva.nl

Trial Manager AMC: M. Spiering, MSc. Head Clinical Trial Office (CTO)
Academic Medical Center, University of Amsterdam
Department of Hematology; F4-224
Phone: +31-205665785
E-mail: m.spiering@amc.uva.nl

Registration: Academic Medical Center
Trial Office Department of Hematology
Email: hemat.trial@amc.nl
Phone: +31-205665950

SUMMARY

Rationale:

The oral cavity is a common site of complications related to hematopoietic stem cell transplantation (HSCT). Oral complications have a negative impact on quality of life (QoL) and treatment outcomes. The knowledge on risk factors and clinical characteristics, burden of illness, and consequences of oral complications related to HSCT is inadequate. This lack of clarity is reflected in a lack of effective oral management regimens. The present observational international multicenter study with the acronym "ORA-STEM" will be performed to attenuate this gap of knowledge. In addition, there is an urgent need to obtain a better understanding of the influence of the oral environment (e.g., pre-transplant dental pathologies, oral microbiome, salivary output and proteome) on the pathogenesis of oral complications. Therefore, the Dutch centers (AMC and RUMC) will perform a side study involving Dutch subjects only aimed to further unravel the role of the oral ecosystem. We anticipate that the results of ORA-STEM as well as our side study will provide a scientific base for the development of individualized preventive strategies.

Main objectives:

- To Identify the incidence, severity and temporal relationships of subjective and objective oral complications related to type of conditioning regimen in HSCT recipients
- To determine which oral complications can predict negative clinical and economical outcomes and reduced QoL
- To explore whether genetic polymorphisms in candidate genes demonstrate an increased risk for the development of severe oral mucositis (OM) and graft versus host disease (GVHD)

- To assess how oral complications are related to other confounding variables than the conditioning regimen

Additional objectives of the Dutch side study:

- To determine whether a less diverse oral microbiome is associated with increased incidence, severity, and duration of OM, and increased incidence and severity of acute and chronic oral GVHD
- To assess whether decreased salivary output and an aberrant salivary proteome are associated with increased incidence, severity, and duration of OM and increased incidence and severity of acute and chronic GVHD
- To assess selecte
- To determine whether pre-existent periodontal disease increases the incidence, severity and duration of OM and incidence and severity of acute and chronic oral GVHD
- To assess whether chronic oral GVHD is associated with increased incidence and activity of dental caries

Study design: Prospective observational multicenter study

Study population: Patients ≥ 18 years diagnosed with a malignancy who will receive full intensity or reduced conditioning therapy followed by autologous or allogeneic HSCT

Main study parameters/endpoints:

- Conditioning regimen (full intensity versus reduced intensity regimens with or without total body irradiation), subjective (e.g., pain, xerostomia, taste changes, dysphagia) and objective oral complications (e.g., mucositis, infections, bleeding, gingivitis, periodontitis, dental caries, osteonecrosis and GVHD); see appendix for assessment details
- Oral complications predictive for decreased QoL (OMDQ, EORTC-QLQ-C30) and negative clinical and economic outcomes (e.g., infections, use of antibiotics and opioid analgesics, parenteral nutrition, additional hospital visits, prolonged hospital stay, death)
- Genetic polymorphisms in candidate genes (microarray analysis of salivary DNA samples) predictive for severe OM and GVHD
- Confounding variables (demographics, cancer diagnosis, therapy, antimicrobial prophylaxis, Keratinocyte growth factor, immunosuppression, local oral care program, non-oral signs/symptoms, nausea/vomiting, diarrhoea, fever, weight, blood values)

Additional study parameters/endpoints of the Dutch side study:

- Alterations of the oral microbial community (identified by open-end next generation sequencing) and in the salivary flow and proteome (MALDI-TOF, 2D-SDS PAGE and Mass Spectometry) in HSCT recipients developing severe OM (WHO>2) and oral GVHD compared with those who do not.
- Periodontal disease (measured by Plaque Index, Gingival Index, and Periodontal Pocket depth) predictive for developing severe OM and GVHD
- Chronic oral GVHD and incidence and activity of dental caries (DMF-T and ICDAS-II score)

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:
The burden for patients of this observational study is mild. Study procedures include oral examinations that are part of standard care and collecting of oral rinsing and salivary samples and completion of questionnaires. There are no direct risks or benefits risks for participating subjects in this study. The anticipated knowledge gained from this study will help to improve future supportive care protocols for HSCT recipients.

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Principal Investigator Name (print) ……..

Principal Investigator Signature …… Date ……..

And

Principal Investigator Name (print) ……..

Principal Investigator Signature …… Date ……..

PROTOCOL AGREEMENT PAGE

I agree to conduct the Clinical Trial in accordance with the current protocol and comply with its requirements, subject to ethical and safety considerations.

Institute ……..

Investigator Name (print) ……..

Investigator Signature …… Date ……..

LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**ABR form, General Assessment and Registration form, is the application form that is required for submission to the accredited Ethics Committee (In Dutch, ABR = Algemene Beoordeling en Registratie)**
ACTA	**Academisch Centrum Tandheelkunde Amsterdam**
AE	**Adverse Event**
AMC	**Academic Medisch Centrum**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects**
cGVHD	**Chronic Graft versus Host Disease**
CMC	**Carolinas Medical Center**
CRF	**Clinical Research Form**
CRP	**C-Reactive Protein**
CT	**Chemotherapy**
DAMPs	**Damage-Associated Molecular Patterns**
DSMB	**Data Safety Monitoring Board**
FIC	**Full Intensity Conditioning**
GCP	**Good Clinical Practice**
GI	**Gastrointestinal**
GVHD	**Graft Versus Host Disease**
HSCT	**Hematopoietic Stem Cell Transplantation**
IC	**Informed Consent**
ICDAS-II	**International Caries Detection and Assessment System II**
METC	**Medisch Ethische Toetsing Commissie (METC)**
OM	**Oral Mucositis**
PI	**Principal Investigator**
PTL	**Platelets**
QoL	**Quality of Life**
RIC	**Reduced Intensity Conditioning**
RT	**Radiation Therapy**

RUMC	**Radboud University Medical Centre**
(S)AE	**(Serious) Adverse Event**
SNPs	**Single Nucleotide Polymorphisms**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
WBC	**White Blood Cell Count**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**
WHO	**World Health Organization**
WMO	**Wet Medisch-wetenschappelijk Onderzoek met Mensen**

1. INTRODUCTION AND RATIONALE

Despite major advances in transplant medicine, significant challenges remain that limit more widespread application of HSCT (1). Full intensity conditioning (FIC) regimens are associated with significant morbidity and mortality, and although reduced intensity conditioning (RIC) regimens have decreased treatment related mortality, major complications still occur. Mucositis, dysphagia (swallowing problems), dysgeusia (taste alterations), infection, pain, xerostomia and GVHD are amongst the diverse spectrum of oral side effects in HSCT recipients (2;3), and are associated with significant morbidity, decreased QoL, and costs. Unfortunately, effective management options are scarce.

To fully understand which oral complications from cancer therapies are important targets for prevention and better management, it is important to understand the burden of illness from cancer and cancer therapies. Numerous preventive care protocols have been proposed to minimize oral complications. Unfortunately, these protocols are rarely evidence-based and often rely on "expert opinion" or anecdotes. The lack of well-designed, prospective studies is the primary reason for the limitation in preventive and management protocols (4).

Studies indicate that genetic predisposition has a role in oral mucositis (OM) (5). Individual genetic differences accounted for variations in mucositis from a specific chemotherapy regimen. For example, patients undergoing HSCT who demonstrated certain genetic polymorphisms of a folate-metabolizing enzyme experienced more severe mucosal toxicity from methotrexate (6). Microarray analysis of salivary samples before and following chemotherapy will allow us to identify candidate genes that may be important in the pathophysiology or as predictors of the development of OM. Such genes may form the basis for future screening tests to predict the likelihood of OM following treatment with specific conditioning protocols and identify patients who would benefit from targeted preventative therapy.

Disturbed homeostasis of the oral cavity has been suggested to be an important common component of the pathogenesis of oral complications (7;8). A healthy oral ecosystem is maintained by a complex network of a commensal microflora and innate and adaptive immunity (9;10). Studies suggest that an altered oral microbiome (11) as well as decreased salivary defense mechanisms influence the severity of OM (12), and may be linked to the development of oral GVHD (7). There is evidence for a role of specific microorganisms in the pathogenesis of OM and oral GVHD, but studies using open-end state of the art technology are necessary to provide a more comprehensive insight in the contribution of the microbiome.

Whole saliva, a mixture of salivary gland secretions, gingival crevicular fluid, transudating plasma proteins

and products of the microbial flora, contains multiple components of the innate and adaptive immune system (7). Saliva is crucial for maintaining oral homeostasis. Studies exploring the salivary output and defense mechanisms in relation to the development of OM and oral GVHD are warranted.

A poor dental condition has been identified as an environmental factor that may exaggerate OM (13;14) and oral GVHD (15). The influence of the dental condition, particularly that of the periodontium on OM and oral GVHD needs further investigation. Moreover, post-HSCT changes of the oral environment may negatively impact dental health, particularly in the setting of cGVHD (16). Studies directed to salivary changes could provide a better insight in risk factors for dental caries associated with GVHD and may provide guidance for preventive interventions.

Taken together, there is a pressing need for a prospective, observational multicenter study including sufficient numbers of patients to establish the nature, incidence and temporal relationship of oral complications related to various conditioning regimens. In addition, it is mandatory to obtain a better insight in the role of the oral environment in the pathogenesis of oral complications. With a deeper understanding of oral complications and risk factors, individualized oral care regimens to minimize such complications can be formulated and evaluated. Evidence–based management recommendations may lead to better patient outcomes and improved cost-effectiveness.

2. OBJECTIVES

Overall aim

The overall aim of this prospective international observational multicenter study is to establish the nature, incidence and temporal relationship of oral complications related to conditioning regimens for stem cell transplantation and immunologic reactions (mainly GVHD) and to determine what objective and subjective oral complications related to treatment can predict negative clinical and economic outcomes and reduced QoL

Primary objectives:

- To Identify the incidence, severity and temporal relationships of subjective and objective oral complications related to type of conditioning regimen in HSCT recipients
- To determine which oral complications can predict negative clinical and economic outcomes and reduced QoL

Secondary objective:

- To explore whether genetic polymorphisms in candidate genes demonstrate an increased risk for the development of severe OM and GVHD
- To assess how oral complications are related to other confounding variables than the conditioning regimen

Additional objectives of the Dutch side study:

The AMC and RUMC will perform a side study involving Dutch ORA-STEM subjects only, aimed at further unravelling the impact of the local ecosystem on oral complications.

- To determine whether a less diverse oral microbiome is associated with increased incidence, severity, and duration of OM, and increased incidence and severity of acute and chronic oral GVHD
- To assess whether decreased salivary output and an aberrant salivary proteome are associated with increased incidence, severity, and duration of OM and increased incidence and severity of acute and chronic GVHD
- To determine whether pre-existent periodontal disease increases the incidence, severity and duration of OM and incidence and severity of acute and chronic oral GVHD
- To assess whether chronic oral GVHD is associated with increased incidence and activity of dental caries

3. STUDY DESIGN

This prospective international observational multi-center study will be conducted in the United States of America, Sweden, Brazil, Australia, Canada, and Dutch centers: AMC and ACTA (Amsterdam) and RUMC and Faculty of Dentistry RUMC (Nijmegen). The AMC and RUMC will perform an additional side study involving Dutch ORA-STEM subjects only. This observational side study will make use of the assessment points of ORA-STEM to perform additional sampling.

Please see Chapter 5.3 Figure 1 and Table 1 for detailed information on the assessment and sampling time points. We expect to include the last Dutch subject within 3 years after start of the study.

4. STUDY POPULATION

4.1 Population (base)

Patients diagnosed with a malignancy, aged 18 years or older that will undergo autologous or allogeneic hematopoietic stem cell transplantation. The hematology department of the AMC performs approximately 45 autologous and 30 allogeneic HSCTs per year, whereas in in the RUMC around 70 autologous and 60 allogeneic HSCTs are performed yearly.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- 18 years or older
- Admitted at the hematology departments of the AMC or RUMC
- Diagnosed with a malignancy, and planned to receive FIC or RIC, followed by autologous or allogeneic HSCT
- Able and willing to provide written and dated informed consent prior to any study specific procedure

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Patients unable to give written and dated informed consent
- Patients younger than 18 years

4.4 Sample size calculations

Sample sizes were selected to provide acceptable precision for estimates of risk for oral complications, based on a systematic literature review providing baseline data on the prevalence and scope of oral complications from cancer (4). These samples sizes were determined with $\alpha = 0.05$ and differing levels of acceptable absolute precision. Using the most conservative estimate, a total of 254 patients will need to be enrolled to obtain 6% precision for the main oral complications listed in Table 1 (see appendix). For the study on genetic polymorphisms, additional pilot studies have to be made and a power analysis to determine the sample size.

For the side study involving Dutch subjects only:

Studies directed to the oral environment and associations with OM will be performed on multiple myeloma patients treated with high dose melphalan conditioning with subsequent autologous HSCT. Oral environmental factors and associations with GVHD will be studied in matched allogeneic HSCT recipients.

For the power analyses, we used the equation for longitudinal data analyses. As changes in the oral microbiome will be partly the result of yet unknown changes in the oral proteome, it is to be expected that the effect size in the proteome will be at least equal to or stronger than 0.21. This will result in smaller group size. In other words, 48 autologous and 56 allogeneic transplanted patients necessary for the oral microbiome analysis will most probably be sufficient to study the oral proteome.

We anticipate that 40% of the matched allogeneic HSCT recipients will develop acute oral GVHD. With an anticipated correlation between the follow-up measurements of 0.3 (acute oral GVHD vs. no acute oral GVHD), differences in proportion of 0.21, a significance level of 0.05, a power (1-β) of 0.80 and 7 sample moments per patient, we need to include 56 allogeneic transplanted patients. Approximately 50% will develop chronic oral GVHD. Based on the same calculations, we need to include 50 allogeneic transplanted patients; this number will be also enough to answer our research questions related to GVHD and dental health.

5. METHODS

5.1 Study parameters/endpoints

Primary study parameters/endpoints

- Conditioning regimen (full intensity versus reduced intensity regimens with or without total body irradiation), subjective (e.g., pain, xerostomia, taste changes, dysphagia) and objective oral complications (e.g., mucositis, infections, bleeding, gingivitis, periodontitis, dental caries, osteonecrosis and GVHD); see appendix for assessment details

Secondary parameters/endpoints:

- Oral complications predictive for decreased QoL and negative clinical and economic outcomes (e.g., infections, use of antibiotics and opioid analgesics, parenteral nutrition, additional hospital visits, prolonged hospital stay, death)
- Genetic polymorphisms in candidate genes (microarray analysis of salivary DNA samples) predictive for severe OM and GVHD
- Confounding variables (demographics, cancer diagnosis, therapy, antimicrobial prophylaxis, Keratinocyte growth factor, immunosuppression, local oral care program, non-oral signs/symptoms, nausea/vomiting, diarrhoea, fever, weight, blood values)

Additional study parameters/endpoints of the Dutch side study:

- Alterations of the oral microbial community (identified by open-end next generation sequencing) and in the salivary flow and proteome (MALDI-TOF, 2D-SDS PAGE and Mass Spectometry) in HSCT recipients developing severe OM and oral GVHD compared with those who do not.
- Periodontal disease (measured by Plaque Index, Gingival Index, and Periodontal Pocket depth) predictive for developing severe OM and GVHD
- Chronic oral GVHD and incidence and activity of dental caries (DMF-T and ICDAS-II score)

5.2 Study procedures

Study phases:

This study is divided into 7 phases (see Figure 1 and Table 1):

- **Phase 1, 2a and 2b:** pre-transplant assessments (1-8 weeks before HSCT)
- **Phase 3:** clinical assessments in the first weeks after transplantation until resolution of neutropenia or OM requiring hospitalization (up to 6 weeks post-HSCT)

- **Phase 4**: additional visits for acute oral problems requiring urgent care. This will be documented for up to 6 months for the autologous HSCT patients and 12 months for the allogeneic transplant patients. The CRF of phase 4 will be completed in retrospect.
- **Phase 5:** in autologous HSCT recipients an assessment (oral and dental assessment, sampling procedures and questionnaires) will be performed at 100 days, and at 12 months. In allogeneic HSCT this will be performed at day 100, 6, 12 and 18 months.
- **Phase 6:** autologous HSCT recipients will be asked to complete a questionnaire yearly until resolution of oral complaints. In allogeneic HSCT patients an assessment (oral and dental assessment, sampling procedures and questionnaires) will be performed at 12 and 18 months; thereafter a questionnaire yearly until resolution of oral complaints.
- **Phase 7:** If a patient is deceased this will be noted.

Figure 1 Study flowchart

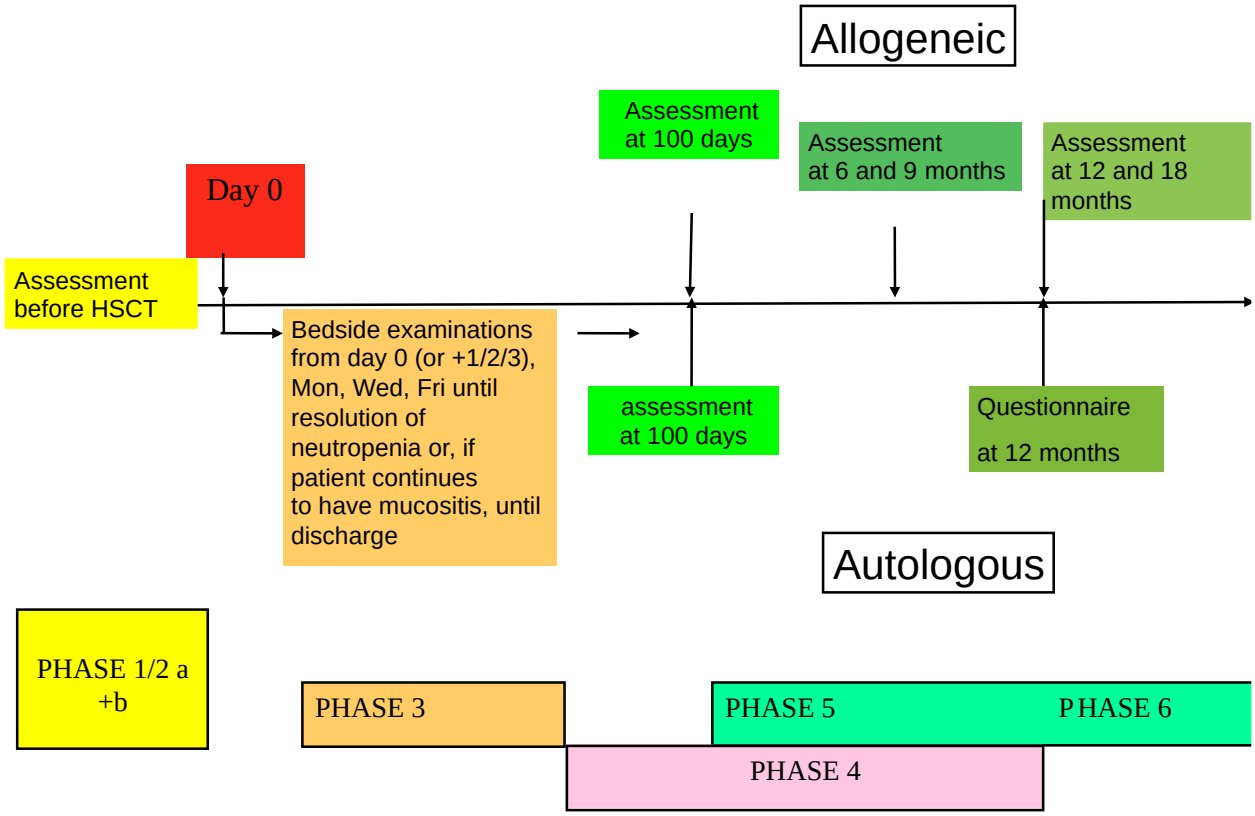

Table 1 Time points of visits and measurements:

Phase:	**1, 2a, 2b**		**3**		**5**								**6**
	Allo	Auto	Allo	Auto	Allo				Auto				Allo
					100d	6m	9m	12m	100d	6m	9m	12m	18m
Dental Health	X	X			X	X		X	X			X	x
Oral Assessment	X	X	X*	X*	X	X	X	X	X			X	x
Saliva Sample for Micro-array	X	X											
Oral rinsing sample	X	X	X*	X*	X	X	X	X	X			X	x
Salivary Output/ sample	X	X	X**	X**	X	X	X	X	X			X	x
Question-naires	X	X	X	X	X	X	X	X	X			X	x
Blood samples[1,]			X	X									
Blood samples [2]	X	X	X**	X**	X	X	X	X	X			X	X

m= months / d=days / Allo= Allogeneic / Auto= Autologous / X* = 3 times weekly / X**= Once weekly

[1] as often as required for standard care in order to evaluate for bacteremia in the neutropenic phase

[2] Blood samples for assessment of the systemic inflammatory response (e.g., DAMPs)

Dental health assessment

The pre-transplant dental health assessment is part of standard care. Caries and periodontal disease will be recorded using validated tools (see Appendix A for details).

If necessary, dental treatment will be performed according to standard treatment protocol of the hospital during the same or subsequent visits. Dental disease left untreated before transplantation due to lack of time or other reasons will be documented.

Oral assessment

Will include subjective and objective evaluation of oral complications (see appendix A for details).

Saliva samples for polymorphism study

We will evaluate if genetic polymorphisms in the candidate genes identified in previous studies performed by Dr M.T. Brennan (International PI) will demonstrate an increased risk for the development of severe OM (WHO grade 3 or 4). A saliva sample will be obtained prior to the start of conditioning therapy, coded and sent anonymously to the department of Oral Medicine, Carolinas Medical Center (CMC), Charlotte North Carolina, USA for storage. We will use the Oragene DNA™ system (DNA Genotek Inc.,Ottawa, Ontario, Canada). All that this requires is for the patients to rinse their mouth with water to clear any food debris before spitting 2 ml of saliva into the container. Once the container is closed, the contained reagents release the DNA from buccal epithelial cells present in the saliva and stabilize it for long-term storage. Following collection, the sample could be stored at room temperature before mailing to the CMC for storage at -20°C until analysis. Approximately 15 candidate genes will be evaluated for polymorphisms that increase the risk for severe OM.

Microbiome

Oral rinsing samples with 10 ml phosphate buffered saline (PBS) will be collected for the newest and high throughput next-generation sequencing (NGS) using specific primers for 16S (bacteria) and 18S (fungi). By using the known databases of both fungi and bacteria, the composition of the microbiome will be determined. Oral rinsing samples will be stored at -80 °C within 30 minutes after collection.

Salivary output and proteome

Patients will be asked to rinse their mouth with water before collecting saliva. Unstimulated whole saliva will be collected for 5 minutes. Whole stimulated saliva is collected after paraffin chewing. Patients spit out all the saliva that accumulates every 30 seconds. Saliva is collected for 5 minutes. The saliva is collected in pre-weighted tubes that are weighted again after saliva collection to quantify the amount of saliva (to determine the flow rate per minute). At this time the pH is measured using pH indicator strips (pH 6.5–10.0; 109543 Merck, Darmstadt, Germany). When the pH is < 6,5 an additional strip with pH range 5.2 – 7.2 will be used (109547 Merck Darmstadt, Germany). After collection the saliva is

immediately frozen at -20 °C and stored at -80°C until analysis. The glycol-protein composition of saliva and overall protein profiles are determined using the open-end techniques 2D-SDS PAGE and Mass Spectrometry.

Questionnaires

EORTC QLQ30, PRO-OMDQ-GVHD and PRO-OMDQ-MUCOSITIS will be used for this trial.

Blood samples

Blood samples assessed as part of standard care in patients with febrile neutropenia and found to be positive for bacteria (defined by recovery from at least one blood culture of any bacterium with the exception of skin commensals such as *Staphylococcus epidermidis* or other coagulase negative staphylococci for which recovery of the same species from at least two blood cultures is required) will be stored at -80°C for potential later comparison with the oral microflora.

At several time points concomitant with salivary sampling (see table 1) 4.5 ml citrate and 4,5 ml EDTA blood will be drawn. These samples will be centrifuged and stored at -80°C for assessment of the native immune response (e.g., Damage-Associated Molecular Patterns; DAMPS) and their potential association with oral complications.

5.3 Premature termination of the study

No premature termination of the study is expected.

5.4 Withdrawal of individual subjects

Participants can withdraw from the study at any time for any reason without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

5.5 Replacement of individual subjects after withdrawal

Patients will only be replaced if withdrawal occurs before phase 4.

6. SAFETY REPORTING

6.1 Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

6.2 Adverse and serious events

No (serious) adverse events are expected from this study.

7. STATISTICAL ANALYSIS

Differences between groups will be analysed by the Student's t-test for independent variables and by a paired t-test for dependant variables. When more than two groups are compared, an analysis of variance (ANOVA) will be performed. Relationships between variables will be calculated with the Chi-square test, Spearman or Pearson correlation, depending on the type of data.

Relationship with data that are measured longitudinally will be analysed by the regression technique Generalized Estimating Equations (GEE) or an ANOVA for repeated measures (GLM for repeated measures).

The output of the microbiome data will consist of Shannon diversity index per individual sample, data ordination plots using the principal component analysis (PCA) on the OTU-table, Bray-Curtis similarity index between the related samples collected at different time points and a robust grouping of the samples per individual time point using spectral clustering (SC) analysis.

Longitudinal changes in salivary proteins will be analyzed with analysis of variance for repeated measures for parametric parameters and Friedman's test for non-parametric parameters for individual salivary proteins. SC analysis will be performed on the baseline values of the final dataset.

In general, a value of $\alpha<0.05$ will be considered significantly different. Analyses will be performed with the statistical programs SAS and SPSS.
Missing data will not be estimated on the basis of other data, so they are defined as missing data in the data set.

8. ETHICAL CONSIDERATIONS

8.1 Regulation statement

This protocol is in accordance with the principles laid down by the 18th world medical assembly (Helsinki 1964) and amendments laid down by the 29th (Tokyo 1975), the 35th (Venice, 1983), the 41st (Hong Kong 1989) and 64th (versie Fortalezea, 2013) World Medical Assemblies.
This protocol is in accordance with laws and regulations of the country in which the study is performed.

8.2 Recruitment and consent

The informed consent document will be used to explain in simple terms, before persons are entered into this study, the nature, scope and possible consequences of the study. The participant will give consent in writing. The signature of the physician and participant must confirm the participant's consent. The investigator is responsible to see that informed consent is obtained from the participant and for obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedure. The signed informed consent forms remain with the investigator.

8.3 Benefits and risks assessment, group relatedness

The expected burden of this study might come from the time patients need to invest to fill out questionnaires and for sampling procedures. The burden of this study may be considered minimal, as study visits will be part of standard care and minimized to extra blood draw during standard venipunctions. There will be no direct risks or benefits for participating subjects, although the oral assessments performed by experienced and trained evaluators may reveal complications (e.g., pain, infections, dental caries in patients with GVHD) at an early stage, allowing early management. The anticipated knowledge gained from this study will help to improve clinical treatment protocols in the future providing a better supportive care regimen in HSCT recipients.

8.4 Confidentiality

Personal information on the patients will be treated confidentially and anonymously according to the 'Wet Bescherming Persoongegevens'. All patient names will be kept secret to anyone other than the

investigator. Participants will be numbered consecutively in the order in which they are included in the study, the next participant receiving the next available number. The number allotted to them during the study will identify patients throughout documentation and evaluation. The participants will be told that all study findings will be stored on computer and handled in strictest confidence formulated in the 'Wet op de geneeskundige behandelovereenkomst' (WGBO).

8.5 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 may be labeled with the patients identifying information (e.g. name, hospital record number)
Samples that are shipped to another facility (e.g. a central laboratory) for a purpose as described in this protocol or for additional scientific research, should be stripped from any identifying information and labeled with a code (trial name or number and patient study number as assigned at enrolment).

8.6 Compensation for injury

As participating in this trial is considered to be without risk, no insurance policy will be obtained to cover the risks of participating in this trial.

9. ADMINISTRATIVE ASPECTS AND PUBLICATION

Eligible patients should be registered before start of treatment. Patients need to be registered at the Clinical Trial Office of the department of Hematology of the Academic Medical Center sending the completed registration form by email (hemat.trial@amc.nl) from Monday through
Friday 09:00 to 17:00 CET. Patient study number will be given and confirmed by email.

9.1 Handling and storage of data and documents

All patient material will be anonymized. Any excess material will be stored to a maximum of 15 years. Only investigators mentioned in this protocol will have access to these samples. The handling of personal data will comply with the Dutch Personal Data Protection Act (in Dutch: De Wet Bescherming

Persoonsgegevens, Wbp) and the privacy regulations of the Academic Medical Center. Personal data will be stored anonymously by means of a code number. Only this number will be used in study documentation, rapports and publications. Only the person who possesses the code key will know the identity of the persons behind the code numbers. Other persons/authorities that are allowed access to the code key are the members of the Independent Ethics Committee of the Academic Medical Center, members of the research team and the Health Inspection. This might be necessary to inspect the accuracy and quality of the study.

9.2 Data monitoring

Data monitoring will be performed by a certified clinical research associate of our institute. The monitor will compare the data entered into the database with the hospital or clinic records (source documents). The nature and location of all source documents will be identified to ensure that all sources of original data required to complete the database are known to the investigational staff and are accessible for verification. At a minimum, source documentation must be available to substantiate: subject identification, eligibility and participation; proper informed consent procedures; dates of visits; adherence to protocol procedures; records of safety and efficacy parameters; adequate reporting and follow-up of adverse events; date of subject completion, discontinuation from treatment, or withdrawal from the study, and the reason if appropriate. Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the database are consistent with the original source data.

9.3 Amendments

Amendments are changes made to the research after a favorable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favorable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the METC 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 METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

9.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the study to the accredited METC 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 study, any problems, and amendments.

9.5 End of study report

The investigator will notify the accredited METC of the end of the study within a period of 90 days. The end of the study is defined as the last patient's visit. In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the PI will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

9.6 Public disclosure and publication policy

It is our intention that the findings of the study will be published in peer reviewed journals and presented at scientific meetings. Furthermore, the results of Dutch side study will form part of 2 PhD theses that will be written and defended.

Authorship

Authors of the main manuscript will include the Principal Investigator, the Co-investigators and, investigators who have included evaluable patients in the trial. Others who have made a significant contribution to the trial may also be included as author, or otherwise will be included in the acknowledgement.

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APPENDIX A

Table 2 Measures of dental caries history and activity

Dental health			
Parameter	Examination	Scoring	Calculation
DMFT index	DMFT measures the prevalence of dental caries.	• The D component scores Decayed teeth • The M component scores Missing teeth due to caries • The F component scores Filled teeth due to caries	The DMFT index can be calculated by summing all the individual items together (D + M + F), with a maximum score of 32.
ICDAS-II	The ICDAS-II will be used to qualitatively assess caries. All surfaces of each tooth present will be scored.	0. Sound 1. First Visual Change in Enamel 2. Distinct Visual Change in Enamel 3. Localized Enamel Breakdown (without clinical visual signs of dentinal involvement) 4. Underlying Dark Shadow from Dentin 5. Distinct Cavity with Visible Dentin 6. Extensive Distinct Cavity with Visible Dentin	Each tooth will be categorized in one of the following categories: - Score 0: sound tooth - Score 1 & 2: early stage decay - Score 3 & 4: established decay - Score 5 & 6: severe decay

DMFT = Decayed Missing Filled Teeth, ICDAS = International Caries Detection and Assessment System

Assessment of dental plaque

Baseline oral hygiene will be measured by the number of tooth surfaces with plaque present. The patient will best chew on a disclosing tablet (erytrosine), then plaque visible with the eye (yes/no) will be registered for four surfaces for each tooth:

- No visible plaque
- 1-20% of surfaces with plaque will be graded as good oral hygiene
- 21-50% is intermediate and
- >50% of the surfaces is considered poor oral hygiene

Calculus measurement

- Supragingival calculus (yes/no) on any surface of the tooth. Registered tooth by tooth
- Subgingival calculus (yes/no) identified clinically and/or on dental radiographs on any surface of the tooth. Registered tooth by tooth.

The % of teeth with the presence of calculus will be reported.

Periodontal pocket depths and Bleeding on Probing

- Full pocket depth index will be registered on four surfaces on each tooth at the dental clinic.
- The number of teeth with at least one pocket >5 mm (deep pockets) will be registered.

Bleeding on Probing (BOP):

- Bleeding will be noted (Y/N) with each tooth.
- The percentage of teeth with the presence of bleeding on probing will be reported.

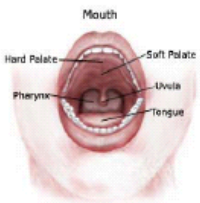
Table 3 World Health Organization (WHO) score for oral mucositis

WHO score for oral mucositis	
Grade 0	No findings or erythema without soreness
Grade 1	Sores present with or without erythema
Grade 2	Ulcers present but able to take solid food
Grade 3	Ulcers present and only able to take liquids
Grade 4	Ulcers present and not able to take anything orally

Table 4 Oral Mucositis Assessment Score (OMAS)

	Grade 0	**Grade 1**	**Grade 2**	**Grade 3**
R=Redness	None	Moderate	Severe	-
U=Ulcerations or pseudomembranes	None	<1 cm^2	1-3 cm^2	>3 cm^2

Table 5 The NIH oral cGVHD Activity Assessment

Mouth	Mucosal change	No evidence of cGvHD		Mild		Moderate		Severe	
	Erythema	None	0	Mild erythema or moderate erythema (< 25%)	1	Moderate (≥ 25%) or severe erythema (< 25%)	2	Severe erythema (≥ 25%)	3
	Lichenoid	None	0	Hyperkeratotic changes (< 25%)	1	Hyperkeratotic changes (25%–50%)	2	Hyperkeratotic changes (> 50%)	3
	Ulcers	None	0	None	0	Ulcers involving ≤ 20%	3	Severe ulcerations (> 20%)	6
	Mucoceles*	None	0	1-5 mucoceles	1	6–10 scattered mucoceles	2	Over 10 mucoceles	3
				*Mucoceles scored for lower labial and soft palate only				Total score for all mucosal changes	