
**PROTOCOL**

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EudraCT number : 2012-004915-30
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By my signature, I agree to personally supervise the conduct of this study in my affiliation 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.
# Scheme of study

Steroid-refractory Acute Graft-versus-Host Disease Grade II-IV (with gut and/or liver involvement)

![Diagram showing the scheme of study]

**Arm A**
- MMF + Placebo
- Day 1 and Day 8

**Arm B**
- MMF + MSC $2 \times 10^6$/kg i.v.
- Day 1 and Day 8

**Evaluation**
- Day 29

Off protocol treatment
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3 Synopsis

Rationale

Steroid-refractory acute Graft-versus-Host Disease (GvHD) is a potentially lethal disorder. A variety of second line immunosuppressive agents have been investigated but no optimal treatment has emerged. There is therefore a need for novel treatment strategies. Mesenchymal stromal cells (MSC) exhibit immunomodulatory properties and a recent pilot study suggests a response rate of 70% in steroid-refractory patients. In the present randomized study the efficacy and safety of MSC treatment will be further studied in patients with severe steroid-refractory acute GvHD.

Study objectives

Primary objective

To improve the response rate to treatment of acute GvHD grade II-IV (with gut and/or liver involvement) by early addition of MSC to standardized second line treatment.

Secondary objectives

- To study the safety of MSC addition to second line treatment
- To assess the overall survival
- To assess the progression-free survival
- To reduce the time required for continued pharmacological immune suppression
- To assess the incidence of severe bacterial, viral and fungal infections
- To assess the incidence and severity of chronic GvHD
- To evaluate the quality of life of patients treated with MSC in comparison with controls up to two years after MSC treatment
- To develop a score by means of clinical and laboratory parameters that allows for identification of patients with acute GvHD that will respond to MSC treatment
Study design
This is a prospective, multi-center, placebo-controlled, randomized, double-blind phase III trial.

Patient population
All patients who underwent an allogeneic hematopoietic stem cell transplantation (HSCT) for malignant or non-malignant (immune-) hematological disorders, developing grade II-IV acute GvHD (with gut and/or liver involvement) either directly after HSCT or following treatment with donor lymphocyte infusion (DLI), and display progressive disease or mixed response following 5 days of consecutive systemic treatment with steroids at a dose of 2 mg/kg and a calcineurin-inhibitor, or stable disease following 10 days of consecutive systemic treatment with steroids at a dose of 2 mg/kg and a calcineurin-inhibitor.

Intervention
Eligible patients will be randomized to either standardized second line treatment only, consisting of mycophenolate mofetil (MMF) in combination with a placebo for MSC infusion, or MMF in combination with MSC at a dose of 2 x10^6 MSC per kg bodyweight IV. The first gift of MSC or placebo will be administered the day following randomization. The second gift will be administered 7 days after the first gift.
In addition, all patients will continue systemic treatment with steroids and a calcineurin-inhibitor.

Duration of treatment
The expected maximum duration of treatment according to the study protocol will be 4 weeks.

Patients will be evaluated at entry, at day 8, 15, 22 and 29, after 6 weeks, and at 2, 3, 6, 12, 18, and 24 months after randomization.
Subsequently patients will be followed until 10 years after registration.

Target number of patients 150
Expected duration of accrual: 2.5 years

Study endpoints

Primary endpoint:
Proportion of patients responding to treatment of acute GvHD grade II-IV (with gut and/or liver involvement) at day 29.

Secondary endpoints
1. Overall survival
2. Progression-free survival
3. Duration of acute GvHD response
4. Time without systemic immunosuppression
5. Cumulative incidents of non-relapse mortality
6. Adverse events
7. Incidence of chronic GvHD
8. Quality of life
9. Immune reconstitution including monitoring of absolute T-cell subsets, B-cells, NK-cells as well as biomarkers of acute GvHD (see appendix G)

Benefit and nature and extent of the burden and risks associated with participation

There is no established treatment for steroid-refractory acute GvHD. Treatment with bone marrow-derived expanded MSC may induce responses of acute GvHD. Based on pilot studies, the infusional toxicity is expected to be minimal and restricted to fever. Potential adverse events, that have not been observed in pilot studies so far, include suppression of immune responses increasing risk of infection and relapse of malignant disease. Moreover, MSC mediated immunosuppression could result in an increased rate of relapse. These potential risks, however, are balanced by the expected benefit of MSC therapy namely resolution of GvHD with its known high mortality rate both from GvHD itself and from the opportunistic infections associated with the current immunosuppressive GvHD treatment strategies.
Planned interim analysis and DSMB (if applicable)

Two interim analyses will be planned after inclusion of 33% and 67% of patients.

A DSMB will be installed.
# 4 Investigators and study administrative structure

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5 Introduction and rationale

Allogeneic hematopoietic stem cell transplantation (HSCT) is a successful therapy in use since the 1960's and a proven cure for patients suffering from hematological disorders as well as immunodeficiencies and metabolic disorders. For many of these patients HSCT is the only curative treatment option.

Despite advances in pre-transplant immunosuppression, donor human leukocyte antigen (HLA) typing methods (and thus donor selection), and donor lymphocyte infusion (DLI) protocols, Graft-versus-Host Disease (GvHD) remains a significant cause of transplant-related mortality and morbidity.1-3 Early effective intervention results in better longterm outcome and quality of life (QoL). First line treatment consists of often protracted immunosuppressive therapy with corticosteroids. Steroid-refractory acute GvHD has a high mortality rate and surviving patients often develop chronic GvHD, which reduces life expectancy, performance and quality of life. Therefore, effective strategies are needed for the treatment of steroid-refractory acute GvHD.

5.1 Graft-versus-Host-Disease

Pathophysiology of GvHD

GvHD can be described as a three-phase process.1 First, damage to host tissue, as occurs with the conditioning of the patient, induces the release of inflammatory cytokines, including IL-1, TNF-α, and interferon-γ.4,5 Next, triggering and activation of donor-derived T-cells by inflammatory cytokines and recipient- and donor-derived antigen-presenting cells (APC), results in the production of IL-2 and interferon-γ (a so-called Th1 response). IL-2 controls and amplifies the allogeneic immune response.6 Finally, the effector phase is characterized by activated donor T-cell mediated cytotoxic damage against host cells through Fas-Fas ligand interaction, perforin-granzyme and TNF-α.7-9 The latter plays a central role in the pathophysiology, stimulating cytokine production (IL-1, IL-6 IL-10, IL-12, and TNF-α). This dysregulation leads to the clinical manifestations of GvHD.1,5,10 Recent studies have also implicated a role for residual host APC in the initiation phase of GvHD and their localization may be relevant to organ-specific manifestations of GvHD.11-13 Their precise role in the clinical setting is, as yet, not determined.

Manifestations of acute and chronic GvHD

The incidence of GvHD following HSCT ranges from 10-80% of patients dependent upon the risk factors present.1 GvHD is often defined as acute when occurring up to 100 days post HSCT and as chronic thereafter. This definition may be inadequate in light of subsequent immune-modulation following HSCT e.g. cessation of cyclosporin A (CsA) and DLI infusions to induce Graft-versus-Leukemia (GvL) effect.1 Recent revision of the grading of acute and chronic GvHD has been
published to reflect the changes in recent clinical observation i.e. symptoms of acute GvHD presenting > 100 days post HSCT. 

Acute GvHD predominantly affects the skin, upper and lower gastro-intestinal tract, liver and occasionally the eye and oral mucosa. Clinical grading is determined by the site and severity of the manifestation (Appendix A). 

Chronic GvHD mainly affects the skin, eye and GI tract, clinically manifesting as a scleroderma-like illness. Occasionally chronic progressive restrictive lung damage may occur. Chronic GvHD is classified as either limited or extensive depending upon the organ systems involved (Appendix A). Biopsy of involved tissues, although lacking sensitivity, may be helpful in confirming the diagnosis when positive, especially if the signs are relatively non-specific. For instance, patients treated successfully with MSC for gastro-intestinal tract GvHD may exhibit persistent diarrhea, related to epithelial damage (villous atrophy and consequent gastro-intestinal malabsorption) rather than persistent GvHD. 

GvHD and quality of life

Acute and chronic GvHD has a robust negative impact on QoL. 6 of 7 studies investigating the association between acute and chronic GvHD and QoL have reported a significant negative relationship. In these studies both forms of GvHD were shown to be associated with worse physical functioning, role functioning, social functioning, mental health, general health and overall QoL. A recent study showed patients with moderate to severe chronic GVHD to have mean physical and mental component scores (SF-36) comparable with those previously reported for systemic sclerosis, systemic lupus, erythematosus, and multiple sclerosis (physical) and depression (mental). The presence of acute and/or chronic GvHD appears to be the strongest predictor of reduced QoL and impaired functional status following HSCT, as self-reported QoL in HSCT survivors without GVHD tends to be very similar to that in comparison groups. Importantly, patients with a history of chronic GvHD in whom GvHD has resolved, have as good a QoL as those with no history of chronic GvHD. These data underline the importance of adequate GvHD treatment for QoL.

Treatment of acute GvHD

The initial management of acute GvHD consists of high dose corticosteroids, which may be combined with calcineurin-inhibitors such as CsA and tacrolimus. The majority of centers utilize methyl prednisolone at 2.0 mg/kg/day. Approximately 50% of patients improve with this treatment. When there is no response to high dose corticosteroids after 7 days, or when there is progressive disease after 72 hours, acute GvHD is considered steroid-refractory. This group of patients has a dismal prognosis. As recent as 2011, mortality rates were reported up to 87% at 2 years follow-up for steroid-refractory patients. The major causes of death are GvHD and infections, not in the least due to the immunosuppressive GvHD treatment regimen.
There is presently no consensus as to the salvage treatment in steroid-refractory acute GvHD. Numerous agents have been reported as second line treatment either alone or in combination and continue to be evaluated.\textsuperscript{23,24} Arai et al. have reported the use of ATG in a population of 69 steroid-refractory patients but only 4 patients survived.\textsuperscript{25} Similar results were reported by Khoury et al. and Remberger et al.\textsuperscript{26,27} Carpenter et al. evaluated anti-CD3 (visilizumab) in a phase 2 multicenter trial, showing a response rate of 32% and an overall survival of 32% at 6 months.\textsuperscript{28} Monoclonal antibodies have been used to target the IL-2 receptor CD25 (daclizumab, basiliximab, and inolimomab). In single center trials, response rates ranged from 58% to 83%, and survival ranged from 30% to 53%. However, responses occurred mainly in patients with low grade disease of either skin or intestinal tract, and there was a high incidence of severe virus and fungus infections.\textsuperscript{29,32} Studies of an IL-2-diphteria toxin fusion product (Denileukin difitox) yielded similar results.\textsuperscript{33,34} In another approach, TNF-\(\alpha\) production was targeted using infliximab with response rates of 59% to 50% and overall survival of 38% to 41%.\textsuperscript{35,36} Of the chemotherapeutic modalities, mycophenolate mofetil (MMF) has been the most extensively studied. Response rates ranged from 30% to 67% and overall survival from 33% to 76% in 3 studies that also included chronic GvHD patients.\textsuperscript{37-39} There have been very few studies comparing different treatment modalities in acute GvHD. In a recent observational study Xhaard et al. reported the results of 93 patients treated with either MMF, inolimomab or etanercept for second line treatment of acute steroid-refractory GvHD. Overall response-rate for MMF was 55% as compared to 35% (inolimomab) and 28% (etanercept), but there was no significant difference in overall survival (30% at 24 months).\textsuperscript{40} In another study of 180 patients comparing MMF, etanercept, denileukin difitox, or pentostatin plus corticosteroids for first line treatment of acute GvHD, the MMF arm showed the highest response rate (60% versus 26%, 53% and 38%) and overall survival (64% versus 47%, 49% and 47%), and the lowest incidence of severe infections (44% versus 48%, 62% and 57%).\textsuperscript{41} Many centers nowadays use MMF as second line treatment for steroid-refractory acute GvHD. However, a significant proportion of patients receives MMF for GvHD prophylaxis and is thus no longer candidates for MMF treatment as salvage therapy.

There is a dire need for new and better treatment modalities for steroid-refractory GvHD. Since 2006, several studies have indicated that third-party mesenchymal stromal cells (MSC) might be an effective therapy for steroid-refractory GvHD. The hypothesis of action is that MSC have immunomodulatory properties that can diminish or eradicate GvHD.

5.2 Mesenchymal Stromal Cells

The bone marrow stroma contains, in addition to hematopoietic stem cells, a population of pluripotent marrow stromal cells, capable of multi-lineage differentiation into fibroblasts, osteoblasts, adipocytes and chondrocyte progenitors.\textsuperscript{42-44} This heterogeneous population of cells provides growth factors, cell-
to-cell interactions, and matrix proteins that play a role in the regulation of hematopoiesis. In 2005, the term “multipotent mesenchymal stromal cells” (MSC) was introduced to describe this population of fibroblast-like plastic-adherent cells. MSC are characterized by the absence of hematopoietic markers (CD45-/CD34-/CD14-) and the expression of a specific pattern of adhesion molecules (CD106+/CD54+/SH2+/SH3+). These molecules mediate homing and differentiation of hematopoietic stem cells.

Although the bone marrow serves as the primary reservoir for MSC, their presence has been reported in a variety of other tissues. These include adipose tissue, periosteum and muscle connective tissue, fetal bone marrow, fetal liver and blood, umbilical cord blood, and cytokine (G-CSF) mobilized peripheral blood. The frequency of MSC in these sources is very low which may explain the contradictory findings of initial researchers. In fetal blood the frequency has been reported to decline with gestational age, from about 1/10⁶ mononuclear cells in first-trimester fetal blood to 0.3/10⁶ MNC in term cord blood. MSC have also been isolated from human amniotic fluid. The phenotype of the culture-expanded amniotic fluid–derived cells was similar to that reported for MSC derived from second-trimester fetal tissues and adult bone marrow.

**Immunomodulatory properties of MSC**

Several studies suggest that MSC play a role in modulation of immune responses. MSC are poor antigen-presenting cells that do not express HLA class II or co-stimulatory molecules. Human bone marrow stromal cells suppress T cell proliferation induced by cellular or non-specific mitogenic stimuli and inhibit the response of naïve and memory antigen-specific T cells to their cognate peptide. In accordance, expanded MSC do not stimulate T cell proliferation in Mixed Lymphocyte Reactions and are able to down regulate allo-reactive T cell responses when added to mixed lymphocyte cultures. Furthermore, human MSC alter cytokine secretion profiles of dendritic cells, naive and effector T cells, and natural killer cells to induce a more anti-inflammatory or tolerant phenotype. The immune modulatory properties of MSC have been recently summarized. As yet, the mechanisms by which MSC modulate the immune system remain unclear. It would appear that TGF-β1 and HGF are consistently involved in the inhibitory effects of MSC on T cells. There is also growing evidence for a key role of the innate immune system.

**Production of MSC**

At present no unique phenotype has been identified that allows the reproducible isolation of MSC precursors with predictable developmental potential. The isolation and characterization of stromal cell function therefore still relies primarily on their ability to adhere to plastic. Standard conditions for expansion of MSC include the presence of serum, in most instances fetal bovine serum. Cell density
is a critical factor affecting the growth of cells. Culture attempts are usually unsuccessful below a critical cell density. The cells can be cultured directly i.e. unmanipulated, following collection or after density gradient separation. The CFU-F limiting dilution assay has been used to determine the frequency in bone marrow. A number of markers are expressed on MSC and some of these have been used to enrich MSC from populations of adherent bone marrow stromal cells.\(^{62}\)

Recently, techniques have become available to isolate and culture mesenchymal progenitors and to manipulate their expansion under defined \textit{in vitro} culture conditions. As a result MSC can be rapidly expanded to numbers that are required for clinical application. The EBMT consortium has reported the feasibility of using a common MSC expansion protocol for the treatment of refractory acute GvHD. This has allowed for the clinical testing of culture-expanded MSC in the context of HSCT.

\textbf{Animal studies}

Most early animal studies focused on the enhancement of long-term engraftment of human cells by co-transplantation with MSC. Almeida-Porada et al. observed that, both during gestation as well as after birth, co-transplantation of human stromal cells into pre-immune fetal sheep resulted in an enhancement of long-term engraftment of human cells in the sheep bone marrow and higher levels of donor cells in the circulation.\(^{63}\) Studies in NOD/SCID mice indicated that co-transplantation of MSC and cord blood-derived stem cells enhanced engraftment of human hematopoietic cells in the bone marrow of NOD/SCID mice.\(^{64}\) In a study on MSC from non-human primates, Bartholomew et al. demonstrated that MSC infusions can suppress lymphocyte proliferation and prolong skin grafts in a baboon.\(^{65}\) In another study, they showed that both allogeneic and autologous MSC home to injured tissues, including the gut, following total body irradiation in baboons.\(^{66}\) In mice, infusion of allogeneic MSC ameliorated lethal GvHD following haplo-identical stem cell transplantation, but only when MSC were administered early and repeatedly.\(^{67}\)

\textbf{Early clinical studies}

Initial phase I studies involving bone marrow-derived MSC showed that MSC could be successfully collected, culture-expanded ex-vivo, and administered to patients with hematological malignancies in complete remission. The infusions contained up to $50 \times 10^6$ MSC and were well tolerated without adverse reactions.\(^{68}\) In a phase I-II clinical trial in patients with breast cancer, autologous and expanded MSC were co-infused with autologous peripheral blood progenitor cells.\(^{69}\) No toxicities were observed related to the infusion of MSC and hematopoietic reconstitution was rapid, suggesting some efficacy of MSC infusion on hematopoietic reconstitution. In another phase I-II study, allogeneic donor bone marrow-derived MSC were co-infused in patients with hematological malignancies undergoing matched sibling stem cell transplantation.\(^{70}\) There was no immediate toxicity following infusion of MSC
and there was a more rapid engraftment and a low incidence of acute GvHD in comparison with historical controls. In a phase I-II multicenter study enrolling children given T–cell depleted HLA-disparate allografts, infusion of MSCs proved to be safe and all patients showed sustained engraftment as compared with a 20% graft failure rate in historical controls. These studies showed MSC infusion to be safe and feasible both in children and in adults.

**Long-term effects of MSC**

Since the first clinical studies, numerous trials have been initiated based on MSC treatment to promote engraftment, to decrease GvHD, to treat autoimmune diseases, and to induce tissue repair, for instance the treatment of refractory penianal fistulas in Crohn’s disease. So far, no short or long term adverse events have been reported. It has been debated whether MSC increase the risk of leukemia relapse or infectious complications due to their immunomodulatory properties. In general, any treatment of GvHD is inherently immunosuppressive and thus increases the risk of both relapse and infections. There is as yet no evidence that MSC are intrinsically better or worse in this regard. While initially risks of ectopic tissue formation and sarcoma development have been a concern, clinical experience so far does not support this possibility. Long-term biological safety remains unknown.

### 5.3 MSC for treatment of GvHD

**Studies of MSC in patients with acute steroid-refractory GvHD**

In 2002, the suppressive effect of MSC in the context of GvHD was first demonstrated in a matched pair analysis of patients receiving 1-2 x 10^6/kg MSC that showed a significantly reduced incidence of both acute and chronic GvHD. Next in 2004, Le Blanc et al. reported the successful treatment of severe steroid- refractory grade IV acute GvHD of the gut and liver in a 9-year old with haplo-identical bone marrow-derived MSC. Following this observation, 55 patients (30 adults and 25 children) with steroid-refractory acute GvHD grade II-IV were enrolled and reported in a phase II multicenter study involving five European centers. The patients received a total of 92 MSC infusions from either an identical related (n = 5), haploidentical (n = 18) or third party allogeneic (n = 69) donor. The median dose of bone marrow-derived ex vivo expanded MSC was 1.4 (range 0.4-9) x10^6 cells/kg. Twenty-seven patients received 1 dose, 22 patients received 2 doses and 6 patients received 3 to 5 doses of MSCs. No differences in response were evident dependent upon donor source. All patients had failed to respond to conventional treatment of steroids, and many had been treated with alternative, sequential immunosuppressive regimens. Just over half of patients had a complete response (children 68%, adults 43%), and 17% had a partial response (children 24%, adults 38%) (p=0.07). with an overall response of 72%. Patients with a complete response (CR) had lower transplant-related mortality 1 year after transplantation than patients with partial or no response (37% versus 72%,...
p=0.002) and they had higher overall survival (53% versus 16%, p=0.018). There were no side-effects during or immediately after MSC-infusions.

These results were corroborated by the recent presentation of the outcome of 37 children receiving MSC infusions for grade III-IV steroid-refractory acute GvHD.76 Complete response was observed in 59% of patients. Transplant-related mortality in this group was 14%, as opposed to 60% transplant-related mortality in partial or non-responders (p=0.005). With a median follow-up of 2.3 years, overall survival was 62%. Interestingly, administration of MSC early after the diagnosis of steroid refractory GvHD (regular practice in the participating centers from 2009 onwards) resulted in more complete response rates and better overall disease free survival. Especially the occurrence of fatal infection was reduced compared to patients who had received MSC later in the course of their treatment, possibly related to the ability to stop pharmacological immune suppression and consequent improved immune surveillance. This translated into a significantly better overall survival for children enrolled in the study after 2009 (93% versus 65%, p<0.05). No increased risk of relapse was evident in long-term survivors. However, despite these results the rates of chronic GvHD were high (46%), albeit there was a trend to more limited than extensive cGvHD in patients successfully responding to MSC treatment.

Following these encouraging results, several non-randomized studies were published describing the effects of MSC-infusion in steroid-refractory acute GvHD (Table 1).

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>age</th>
<th>GvHD</th>
<th>MSC</th>
<th>Response</th>
<th>Survival</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ringdén et al. 200677</td>
<td>8</td>
<td>adults: 6 and children: 2</td>
<td>grade III: 6 grade IV: 2</td>
<td>BM-MSC, third party/haplo/sib</td>
<td>6/8 CR</td>
<td>5/8 OS (2-36 months)</td>
<td>no infusional toxicity</td>
</tr>
<tr>
<td>Fang et al. 200776</td>
<td>6</td>
<td>adults</td>
<td>grade III: 2 grade IV: 4</td>
<td>Adipose-MSC, third party/haplo</td>
<td>5/6 CR</td>
<td>4/6 OS (18-90 months)</td>
<td>no infusional toxicity</td>
</tr>
<tr>
<td>Le Blanc et al. 200878</td>
<td>56</td>
<td>adults: 30 and children: 25</td>
<td>grade II: 5 grade III: 25 grade IV: 25</td>
<td>BM-MSC, third party/haplo/sib</td>
<td></td>
<td></td>
<td>no infusional toxicity</td>
</tr>
<tr>
<td>Von Bonin et al. 200979</td>
<td>13</td>
<td>adults</td>
<td>grade III: 2 grade IV: 11</td>
<td>BM-MSC, third party, platelet lysate-expanded</td>
<td>2/13 CR, 5/13 MR</td>
<td>4/13 OS at median FU 207 days (116-261 days)</td>
<td>no infusional toxicity</td>
</tr>
<tr>
<td>Lucchini et al. 201085</td>
<td>8</td>
<td>children</td>
<td>grade I: 3 grade II: 1 Grade IV: 4</td>
<td>BM-MSC, third party, platelet lysate-expanded</td>
<td>3/8 CR, 2/8 PR, 3/8 NR</td>
<td>OS 5/8</td>
<td>no infusional toxicity</td>
</tr>
<tr>
<td>Prasad et al. 201081</td>
<td>12</td>
<td>children</td>
<td>Grade: III-IV</td>
<td>Prochymal</td>
<td>CR 58%, PR 17%, MR 25%</td>
<td>OS 42% (427-1111 days)</td>
<td>no infusional toxicity</td>
</tr>
<tr>
<td>Pérez-Simon et al. 201182</td>
<td>10</td>
<td>adults</td>
<td>grade II: 2 grade III/IV: 8</td>
<td>BM-MSC, donor/haplo/third party, human serum expanded</td>
<td>1/10 CR, 6/10 PR, 3/10 NR</td>
<td>OS 1/10 (11 months)</td>
<td>no infusional toxicity; 1 relapse</td>
</tr>
</tbody>
</table>
So far, only one large randomized placebo-controlled trial evaluating MSC in steroid-refractory acute GvHD has been performed. This company-sponsored trial focused on Prochymal®, a commercial preparation of MSC. The results have not been published in peer-reviewed journals. An abstract presented at the annual meeting of the European Group for Blood and Marrow Transplantation suggested that the primary endpoint had not been reached, but that response rates were better in subgroups of patients with GvHD of gut and/or liver, and in children. No difference in incidence of infection, recurrence of malignancy, or adverse event-related discontinuation between the Prochymal®-arm and placebo-arm was described. Despite the absence of peer-reviewed data, Prochymal® has recently been registered for the treatment of steroid-refractory acute GvHD in Canada and New Zealand.

5.4 Rationale of the study

All of the above observations warrant a randomized placebo-controlled trial of MSC infusions as second line treatment for steroid-refractory GvHD.

In light of the available phase II toxicity and feasibility data in patients with steroid-refractory acute GvHD with gut and/or liver involvement, we hypothesise that early intervention with second line therapy plus MSC combined with standard dose steroids will be superior in outcome, survival and QoL compared to second line therapy and steroids alone. We hypothesise not only will there be a higher response rate but that the time to discontinue pharmacological immune suppression will be shorter, resulting in less infectious complications.

On the basis of the earlier study by Le Blanc et al., we have chosen to give 2 MSC infusions in the investigational treatment arm in addition to standardized second line treatment. As yet little is known regarding optimal dosing of MSC, but most experience has been obtained with doses ranging from 1.4 to 2 x 10⁶ MSC/ kg, the latter of which we will adhere to in this study. In light of the often rapidly progressive nature of GvHD culturing related donor MSC in time for this clinical application is not feasible. Our and others experience suggests that MSC obtained from third party donors can be isolated, expanded, stored and safely administered to non-related recipients. Next to MSC/placebo infusions and standardized second line treatment, steroid treatment and a calcineurin-inhibitor will be continued until tapered. It was previously shown that MSC and CsA exert a synergistic suppressive effect on allo-antigen specific cytotoxic T cells, so we do not expect a detrimental interaction
between MSC and calcineurin-inhibitors. Opportunistic infections remain a matter of concern in this patient population.\textsuperscript{85}

While there is no consensus as to second line treatment in steroid-refractory acute GvHD, we have chosen MMF as standardized second line treatment for both the investigational and the placebo arm because it has shown consistently good response rates (as discussed previously) and is currently the second line treatment agent of choice in many transplant-centers. In consequence, patients pretreated with MMF prophylaxis are excluded from enrolment in this study as the placebo arm’s standardized second line treatment may not offer these patients the best possible salvage therapy. We have selected MMF over Myfortic\textsuperscript{®} because there is little clinical experience with Myfortic\textsuperscript{®} in children.

For our primary endpoint we have chosen the response rate on day 29 (where 1\textsuperscript{st} MSC infusion is day 1). A higher response rate is expected to translate into improved overall survival. Overall survival and QoL are very important secondary endpoints that will be assessed after 2 years follow-up. In the study by le Blanc et al.\textsuperscript{75} the median time from first infusion to response was 18 days (range 3-63 days). The initial follow-up is capped at day 29 to allow treating physicians the option of exploring other therapies in the case of treatment failure, a potentially life-threatening situation. The short follow-up required for our primary endpoint ensures optimal versatility and allows for early trial modifications if considered necessary to optimize patient safety. Following day 29, all treatment modalities, including experimental therapies, are allowed with the exception of MSC. Children < 18 years may receive open label MSC as salvage therapy but only after day 29. We have chosen to make this exception for children because the clinical evidence for MSC in children is stronger than in adults and also because the use of MSC infusions in children with steroid-refractory acute GvHD has been approved by the regulatory authorities both in Canada and in New Zealand. In clinical practice it is therefore no longer acceptable to withhold MSC treatment from this patient category. The study will remain blinded until 2 years after inclusion of the last patient as knowledge of the randomization arm may affect the treating physicians’s future treatment decisions. This way, the important outcomes of survival, QoL and adverse events can still be compared at 2 years follow-up in a randomized setting comparing best local care including MSC therapy versus best local care. Patients will thereafter be followed up until 10 years after randomization to monitor for late adverse events that could possibly be MSC related.

6 Study objectives

Primary objective
To improve the response rate to treatment of severe steroid-refractory acute GvHD grade II-IV (with gut and/or liver involvement) by early addition of MSC to standardized second line treatment.

Secondary objectives
- To study the safety MSC addition to standardized second line treatment
- To assess the overall survival
- To assess the progression-free survival
- To reduce the time required for continued pharmacological immune suppression
- To assess the incidence of severe bacterial, viral and fungal infections
- To assess the incidence and severity of chronic GvHD
- To evaluate the quality of life of patients treated with MSC in comparison with controls up to two years after MSC treatment
- To develop a score by means of clinical and laboratory parameters that allows for identification of patients with severe acute GvHD that will respond on MSC treatment

7 Study design

This is a prospective, randomized, double-blind, placebo-controlled, multi-center phase III trial. Patients that underwent an allogeneic HSCT for malignant or non-malignant (immune-) hematological disorders and developed grade II-IV acute GvHD (with gut and/or liver involvement) not responding to systemic treatment with steroids and a calcineurin-inhibitor, will be randomized to receive either standardized second line treatment only, consisting of mycophenolate mofetil (MMF) in combination with placebo, or MMF in combination with MSC at a dose of $2 \times 10^6$ MSC per kg bodyweight IV. MSC or placebo will be administered 1 day and 8 days following randomization. The expected maximum duration of treatment according to the study protocol will be 4 weeks. Details of all treatments (dose and schedule) are given in paragraph 9.

8 Study population

8.1 Eligibility for registration/randomization

Patients that underwent an allogeneic HSCT for malignant or non-malignant (immune-) hematological disorders and developed grade II-IV acute GvHD (with gut and/or liver involvement) either directly after HSCT or following treatment with DLI not responding to systemic treatment of at least 5 consecutive days, are eligible for randomization.
For the purpose of this study, systemic treatment should have consisted of 2 mg/kg steroids intravenously in combination with a calcineurin-inhibitor at therapeutic trough levels. The calcineurin-inhibitor of choice is cyclosporine A (CsA) with trough levels targeted to the upper therapeutic range (200-400 µg/L using the immuno-assay and 200-300 µg/L using the HPLC). Patients on a prophylactic dose of CsA prior to development of acute GvHD should have their CsA dosage increased to meet the trough levels described above if necessary. Patients who were on tacrolimus prior to development of acute GvHD can continue tacrolimus instead of CsA at a dose based on trough levels targeted to 10-20 µg/L in adults and 5-15 µg/L in children.

Patients with mixed response or progressive disease after 5 days of consecutive systemic treatment with steroids and a calcineurin-inhibitor may be randomized from the 5th day of treatment until the 7th day of treatment. Patients with stable disease after 10 days of consecutive systemic treatment with steroids and a calcineurin-inhibitor may be randomized from the 10th day of treatment until the 12th day of treatment. In addition, patients with a partial response of maximal 1 grade after 5 days of consecutive systemic treatment with steroids and a calcineurin-inhibitor, but persistence of or progression to GvHD grade II-IV after 10 days of consecutive systemic treatment with steroids and a calcineurin-inhibitor without intercurrent steroid tapering may also be randomized from the 10th day of treatment until the 12th day of treatment. (For acute GvHD response criteria see Appendix B)

All patients must be randomized before start of treatment and must meet all of the following eligibility criteria.

### Inclusion criteria

- Grade II-IV acute GvHD with gut and/or liver involvement, confirmed by histology of involved tissues (in case of gut and liver involvement histology of either one of these tissues is considered sufficient); *N.B. if the patient is otherwise eligible but histological confirmation at randomization is lacking, the principal investigator should be contacted.*

- Non-responsive to treatment with steroids and a calcineurin-inhibitor defined as:
  - progressive disease or mixed response after 5 days of consecutive systemic treatment with steroids at a dose of 2 mg/kg and a calcineurin-inhibitor at therapeutic trough levels.
  - stage 4 GvHD of gut and/or liver and deterioration of clinical parameters (gut) or increase of serum total bilirubin levels in μmol/L (liver) after 5 days of consecutive systemic treatment with steroids at a dose of 2 mg/kg and a calcineurin-inhibitor at therapeutic trough levels.
  - stable disease after 10 days of consecutive systemic treatment with steroids at a dose of 2 mg/kg and a calcineurin-inhibitor at therapeutic trough levels.
  - progressive disease after initial partial response of maximal 1 grade after 10 days of
consecutive systemic treatment with steroids at a dose of 2 mg/kg and a calcineurin-inhibitor at therapeutic trough levels.

See appendix A for response criteria

- Any age;
- Lansky / Karnofsky score of ≥20;
- Signed informed consent by the patient and/or parent(s) or legal guardian(s).

### 8.1.2 Exclusion criteria

- Use of prophylactic MMF, Myfortic or other systemic treatment for acute GvHD ≤ 6 days prior to development of acute GvHD grade II-IV with gut and/or liver involvement;
- Systemic treatment for acute GvHD other than steroids and a calcineurin inhibitor (budesonide is considered a local treatment);
- Previous treatment with MSC;
- Progressive or relapsing malignant disease in case of NHL, HL, CLL, MM, and ≥ 5% blasts in the bone marrow in case of AML, ALL, CML;
- Requiring ventilator or vasopressor support;
- Poor performance not expected to survive 14 days;
- Known seropositivity of HIV, Hepatitis B and C, HTLV;
- Known uncontrolled toxicity for DMSO;
- Known anaphylactic reaction to penicillin or streptomycin;
- Known pregnancy;
- Any psychological, familial, sociological and/or geographical condition potentially hampering compliance with the study protocol and follow-up schedule.

### 9 Treatment

#### 9.1 Treatment following randomization

##### 9.1.1 Treatment schedule

Treatment will be given according to the schedule below. For details see paragraph 9.1.2 and 9.1.3
<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose/day</th>
<th>Route of administration</th>
<th>Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMF</td>
<td><strong>Adults</strong> 3dd 15 mg/kg (maximum 3000 mg/day). Children 2dd 600 mg (body surface &lt; 1.25 mg/m²) 2dd 750 mg (body surface 1.25 – 1.5 mg/m²) 2dd 1000 mg (body surface &gt; 1.5 mg/m²)</td>
<td>i.v. / oral*</td>
<td>From randomization until at least day 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcineurin inhibitor</td>
<td>Through levels targeted to the upper therapeutic range</td>
<td>i.v. / oral*</td>
<td>Continued, until at least day 29</td>
</tr>
<tr>
<td>MSC/placebo</td>
<td>2 x 10⁶ MSC/ kg</td>
<td>i.v.</td>
<td>Day 1 and day 8</td>
</tr>
<tr>
<td>Steroids</td>
<td>2 mg/kg</td>
<td>i.v / oral*</td>
<td>Continued, until 3 days sustained response then reduce (see below)</td>
</tr>
</tbody>
</table>

* Patients may switch to oral medication once diarrhea (if present) has subsided.

Please note that the day of the 1st MSC/placebo infusion is counted as day 1.

### 9.1.2 Standardized second line treatment

On the day of randomization, MMF will be started intravenously. Patients may switch to oral medication once diarrhea (if present) has sufficiently subsided as determined by adequate calcineurin inhibitor trough levels following oral administration. MMF will be dosed according to the schedule in paragraph 9.1.1. MMF levels will not be monitored.

The calcineurin-inhibitor will be continued at doses based on trough levels targeted to the upper therapeutic range (see paragraph 8.1). Trough levels should be monitored at least weekly. MMF and calcineurin-inhibitor should be continued at least until the evaluation on day 29. Dose adjustments due to toxicity will be performed according to the schedule described in paragraph 9.4.1.

Steroids will continue to be administered at a dose of 2 mg/kg until a sustained response of acute GvHD has been observed for at least 3 consecutive days. The steroid dose may then be reduced to 50% and subsequently tapered with 20-30% weekly until stopped. In case of progressive disease during tapering of steroids, the steroid dose may be reverted to 2 mg/kg day.

Start of any other systemic third line GvHD treatment prior to day 29 is considered a major protocol violation in which case the patient goes off protocol treatment.
9.1.3 Investigational treatment

Two doses of MSC or placebo will be administered by intravenous infusion via an in-situ venous catheter or indwelling central line. A dose of MSC consists of $2 \times 10^6$ MSC/kg in a range of $1.5 \times 10^6$ MSC/kg to $2.5 \times 10^6$ MSC/kg (maximum $200 \times 10^6$ MSC) with 10% DMSO. Placebo consists of a 10% DMSO-solution in isotonic solution. Medical staff, patients and family will be blinded to the product containing MSC. (For blinding /unblinding procedure see 15.2 and 15.3.) The two doses of MSC or placebo will be administered with an interval of 7 days. In case of MSC, first and second MSC products will be generated from the same donor.

The first MSC/placebo infusion will be administered on the day following randomization. If this is not feasible, the infusion will be administered on the first possible day, with a maximum delay of 3 days. If contra-indications for MSC/placebo infusion arise in the intervening time, such as a septic episode, the MSC/placebo infusion may be postponed but should still be administered on the first possible day. If the MSC/placebo infusion has been postponed and expediency of MSC/placebo infusions is rendered doubtful by clinical developments, the principal investigator should be contacted.

9.2 Treatment following end of protocol

Patients will be evaluated on day 29, and will go off protocol treatment from this day onwards. Treatment after day 29 is at the discretion of the treating physician. In responding patients the following tapering schedule is suggested.

- Steroids may be tapered until stopped following the schedule described in paragraph 9.1.2.
- After withdrawal of steroids MMF may be tapered in 4 weeks time.
- 4-6 weeks after withdrawal of MMF, the calcineurin-inhibitor may be tapered with 10% per week. If the calcineurin-inhibitor was given prior to start of the treatment protocol as a prophylactic immunosuppressant it must be continued according to its original schedule.

Patients with stable or progressive disease on day 29 may be eligible for third line treatment at the discretion of the treating physician. For children, this may include treatment with MSC up to a maximum of 2 infusions. Stable or progressive disease after day 29 is not considered an indication for breaking the blind on a patient, so that children in this case may receive up to a maximum of 4 infusions with MSC. For adults, MSC infusions as salvage therapy are not allowed, and will be considered a major protocol violation.
9.3 Toxicity

9.3.1 Dose adjustments due to toxicity

*Cyclosporin A*

<table>
<thead>
<tr>
<th>Dose</th>
<th>Creatinin &lt; 125 µmol/L</th>
<th>Creatinin 125-150 µmol/L</th>
<th>Creatinin 150-175 µmol/L</th>
<th>Creatinin &gt; 175 µmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Start at 3 mg/kg i.v.</td>
<td>No adjustment</td>
<td>20% decrease</td>
<td>40% decrease</td>
<td>Stop until creatinin ≤ 175 µmol/L, then administer according to creatinin level</td>
</tr>
</tbody>
</table>

*Mycophenolate mofetil*

If severe refractory diarrhea or overt gastro-intestinal bleeding occur and are suspected to be MMF-related, reduce dose by 50%. In case of persisting symptoms or ≥ CTCAE grade 3 toxicity (see Appendix D), MMF may be temporarily stopped. MMF-toxicity is unlikely if there is no improvement of symptoms within 48-72 hours. In case of grade 4 neutropenia (neutrophils < 0.5x10^9/L), MMF dose should be reduced by 20%.

*Mesenchymal stromal cell product*

For MSC no dose limiting toxicity has been reported up to a cumulative dose of 10 x 10^6 MSC/kg. In the present study two fixed doses of 2 x 10^6 MSC/ kg bodyweight will be administered with a one week interval.

9.3.2 Monitoring of MSC/placebo infusional toxicity

Standard clinical practice for the infusion of DMSO will be followed (see Appendix E). Maximum administered volume of DMSO is 1 ml/kg recipient weight per 24 hours. MSC will be infused intravenously over 30 minutes. Close monitoring of vital signs (temperature, pulse, respiratory rate, blood pressure) will be performed and documented before MSC infusion, every 15 minutes during infusion, and 1 hour after transfusion.

We refer to the investigator’s brochure (IB) for a detailed description of potential MSC/placebo infusional toxicities

9.4 Special precautions and supportive care

Patients with involvement of the gut may be started on budesonide 3dd 3mg. Patients with skin-involvement may benefit from betamethason 0.05% or triamcinolon 0.1% topical administration.
During treatment with high dose steroids all patients should receive fungal prophylaxis, a prophylactic antibiotic, osteoporosis-prophylaxis and a protonpump-inhibitor, according to the local institutional guidelines.
Quantitative CMV-loads should be measured weekly, and treatment for CMV-reactivation should be started promptly according to the local procedures. Additional viral screening (EBV, AdV in children) may be performed according to the local institutional guidelines.

9.5 Investigational Medicinal Product: MSC

The MSC product consists of an allogeneic, bone marrow-derived and ex-vivo expanded MSC suspension. Please refer to the Investigation on Medicinal Product Dossier (IMPD) on bone marrow-derived MSC for detailed information on the MSC production, laboratory details and release criteria.

9.5.1 Summary of known and potential risks

MSC infusion has been used in a wide variety of clinical applications, and has an excellent safety profile. Documented toxicity has been limited to infusion reactions. Theoretical risks include:
- infusion reaction
- toxicity of DMSO
- microbiological contamination of MSC product
- posttransfusion virus and/or prion infection
- ectopic tissue formation
- tumorigenicity

During MSC production and infusion safety measures are incorporated to minimize these risks, as detailed in the IMPD. In vivo toxicity studies in mice and monkeys and tumorigenicity studies with human MSC with a follow-up of 26 weeks have not revealed any short- or long-term safety risks related to the infusion of MSC.
We refer to the IB for detailed information on the MSC safety profile.

9.5.2 Preparation and labeling

The preparation of the MSC product will be performed as detailed in the IMPD according to GMP guidelines. Labeling of the MSC product will be performed according to GMP guidelines. Prior to infusion, the bag containing the MSC product will be placed in a non-transparent overpouch to ensure blinding of the patients and nursing staff. The overpouch will be separately labeled with a label stating the patient name, birth date, hospital identification number, HOVON study number, expiration date, and directions for use.
9.5.3 Storage and handling

The manufacturing process is detailed in the IMPD. The MSC product is cryopreserved in portions of 20 ml (20 x 10⁶ MSC), 50 ml (50 x 10⁶ MSC), and 100 ml (100 x 10⁶ MSC). The MSC product is thawed in the clean room directly prior to administration and is released for immediate infusion. After packaging the product is immediately transported on cold packs (4°C) to the site of administration. The product is estimated to remain stable for several hours. The MSC medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.

9.5.4 Study drug supply

The MSC product will be expanded according to the EBMT developmental committee guidelines for expansion of MSC.

Production of MSC for the trial will take place at the following different sites:
1. LUMC Stem Cell Laboratory (Dr. H. Roelofs)
2. Karolinska Insitute Stem Cell Laboratory (Prof. K. Le Blanc)
3. University Hospital Leuven (Prof. Dr. T. Devos).
4. University Hospital Leipzig (Prof. Dr. D. Niederwieser)
5. University Hospital of Salamanca. (Dr. F.M. Sanchez-Guijo)

The participating MSC producing sites will implement standard operation procedures for bone marrow harvesting, MSC isolation, expansion, cryopreservation, culture reinitiation and harvesting, congruent with the IMPD. The principal investigator will ensure standardization by implementing a stringent monitoring plan including yearly sample circulation.

Trial sites certified to store cryopreserved therapeutic cellular products, and thaw and infuse MSC products, but not certified to produce MSC, will be supplied by the appropriate MSC production site provided that standard operating procedures (SOPs) for receipt (check MSC transport conditions), storage (in case MSC are not immediately prepared and administered), and release (by a pharmacist or qualified person) are in place that have been approved by the principal investigator as well as the trial site's responsible pharmacist or qualified person. For the Netherlands, we refer to the document “HOVON113 study product administration outside of the LUMC”.

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9.5.5 Donor recruitment

MSC for this study will be sourced either from a family or from a non-related (3rd party) donor. Irrespective of the eventual source of MSC, two suitable adult (family) donors will be requested to donate bone marrow for MSC expansion at the time of patient enrolment. In this way, (3rd party) MSC will be available for all eligible patients, irrespective of the time required to culture MSC. However, patient entry into the study is not dependent upon the consent of patient-related donors to donate marrow for MSC culture. The principal investigator will ensure that each MSC producing site will have a standardized and approved protocol for donor recruitment in place.

9.5.6 Site of study drug administration

The MSC product will only be administered at trial sites that are JACIE-accredited and GMP-certified. In addition, SOPs for preparing (thawing MSC, labeling and applying the non-transparent overpouch) and administering MSC (to the named patient) must be in place that have been approved by the principal investigator as well as the trial site’s responsible pharmacist or qualified person. Trial sites that do not fulfil these criteria must transfer patients for the purpose of MSC transfusion as outpatient admissions to the nearest qualified trial site (in the Netherlands: LUMC). For the Netherlands, we refer to the document “HOVON 113 study product administration in the LUMC”.

9.5.7 Drug accountability

At each MSC producing trial site records will be maintained of the MSC product’s delivery to the local trial sites, the use by each patient, problems and irregularities during infusion, the maintenance of the blind, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial patients (if applicable). Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor. Standardized HOVON113 study product preparation and infusion report forms should be sent by the local stem cell laboratory to the MSC production center within 24 hours after MSC infusion for each MSC product infused.

9.5.8 Study drug return and destruction

The bag of the used MSC product should be returned to the local stem cell laboratory for control of the blind and subsequent destruction. Partially used MSC product should not be redispensed to either
the same or another patient, but should be returned to the local stem cell laboratory and should be destroyed.

Unused MSC product should be retained by the local stem cell laboratory until the MSC production center has instructed the investigator on the return or destruction of the product.

9.6 Investigational Medicinal Product: placebo

The placebo product consists of a 10% DMSO-solution in isotonic solution. Please refer to the IMPD for detailed information on the excipients.

9.6.1 Summary of known and potential risk

Known and potential risks are limited to toxicity of DMSO and microbiological contamination of the placebo product. During placebo production the same safety measures are incorporated to minimize these risks as for MSC production.

We refer to the Investigator's Brochure (IB) for detailed information on the placebo safety profile.

9.6.2 Preparation and labeling

The placebo product will be prepared and cryopreserved at the MSC production sites in portions of 20 ml, 50 ml, and 100 ml similar to the cryopreserved MSC product portions. Labeling of placebo product will be performed according to GMP guidelines. Non-MSC producing trial sites will be supplied by the appropriate MSC/placebo producing site as to have sufficient cryopreserved placebo product available on site at all times.

The placebo product will be prepared for infusion by the trial site's local stem cell laboratory according to GMP guidelines. In addition, SOPs for receipt (check placebo transport conditions), storage (in case the placebo is not immediately prepared and administered), release (by a pharmacist or qualified person), preparation (thawing placebo, labeling and applying the non-transparent overpouch) and administration of placebo (to the named patient) must be in place that have been approved by the principal investigator as well as the trial site's responsible pharmacist or qualified person. Prior to infusion, the bag containing the placebo product will be placed in a non-transparent overpouch to ensure blinding of the patients and nursing staff. The overpouch will be separately labeled with a label stating the patient name, birth date, hospital identification number, HOVON study number, expiration date, and directions for use.
9.6.3 Storage and handling

The placebo product is thawed in the clean room directly prior to administration and released for immediate infusion. After packaging the product is immediately transported on cold packs (4°C) to the site of administration. The placebo product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.

9.6.4 Drug accountability

At each placebo producing trial site records will be maintained of the product’s delivery to the local trial sites, the use by each patient, problems and irregularities during infusion, the maintenance of the blind, and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial patients (if applicable). Investigators should maintain records that document adequately that the patients were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor. Standardized HOVON113 study product preparation and infusion report forms should be sent by the local stem cell laboratory to the placebo production center within 24 hours after placebo infusion for each placebo product infused.

9.6.5 Placebo return and destruction

The bag of the used placebo product should be returned to the local stem cell laboratory for control of the blind and subsequent destruction. Partially used placebo product should not be redispensed to either the same or another patient, but should be returned to the local stem cell laboratory and should be destroyed.

Unused investigational medicinal product should be retained until the placebo production center has instructed the investigator on the return or destruction of the product.

10 Study procedures

10.1 Time of clinical evaluations

For all study procedures the day of the 1st MSC/placebo infusion is counted as day 1.

♦ At entry,
At day 8, 15, 22 and 29,
Thereafter at 6 weeks and at 2, 3, 6, 12, 18 and 24 months

All patients will be followed until 10 years after randomization.

10.2 Required investigations for patient care and outcome measures

Required investigations at entry, during treatment and during follow up

<table>
<thead>
<tr>
<th></th>
<th>At entry</th>
<th>Day 8, 15, 22, 29</th>
<th>Follow up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>At 6 weeks, and 2, 3, 6, 12, 18, 24 months&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td>Medical history</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Physical examination</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>GvHD response evaluation&lt;sup&gt;1&lt;/sup&gt;</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
<tr>
<td>Toxicity / Adverse events</td>
<td>x</td>
<td>x</td>
<td>x&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hematology</td>
<td>x</td>
<td>x&lt;sup&gt;2&lt;/sup&gt;</td>
<td>x</td>
</tr>
<tr>
<td>Blood chemistry</td>
<td>x</td>
<td>x&lt;sup&gt;2&lt;/sup&gt;</td>
<td>x</td>
</tr>
<tr>
<td>Viral screening</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Calcineurin inhibitor trough</td>
<td>x</td>
<td>x</td>
<td></td>
</tr>
<tr>
<td>Biopsy of involved tissue</td>
<td>x&lt;sup&gt;3&lt;/sup&gt;</td>
<td>x&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Immunological monitoring And biomarker analysis</td>
<td>x&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>x&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>Quality of Life</td>
<td>x&lt;sup&gt;5&lt;/sup&gt;</td>
<td>x&lt;sup&gt;5&lt;/sup&gt;</td>
<td>x&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Informed consent</td>
<td>x</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Acute GvHD will be evaluated at entry, and at day 8, 15, 22, and day 29, then at 6 weeks, and at 2, 3, 6, 12, 18 and 24 months. Chronic GvHD will be evaluated at entry, at day 29, and at 2, 3, 6, 12, 18 and 24 months.

<sup>2</sup> Hematology and samples for immunological monitoring and biomarker analysis should be obtained at day 1, 2, 8, 9, 15, 22 and 29. Blood chemistry should be obtained at day 1, 8, 15, 22 and 29.

<sup>3</sup> Biopsy of involved tissue need not be repeated at entry if obtained prior to start of systemic steroid treatment, and need only be repeated on day 29 in case of absence of complete response.

<sup>4</sup> Samples for immunological monitoring and biomarker analysis should preferably be obtained at the start of systemic steroid treatment as well.

<sup>5</sup> Quality of Life will be assessed at randomization, at day 29 and at 3, 6, 12 and 24 months during follow-up.

<sup>6</sup> And at relapse of GvHD or of the original hematological malignancy.

<sup>7</sup> Limited to survival status, remission status of the original hematological disease, remission status of GvHD, adverse events.

Medical history

Standard medical history, with special attention to:
- Karnofsky / Lansky performance status (see Appendix C).
- Adverse events.
- Infections.
- Concomitant therapy.

Only at entry:
- Non-hematological medical history.
- Antecedent hematological diseases.
- Previous chemotherapy or radiotherapy.

Physical examination
Standard physical examination, with special attention to:
- Standard physical examination including vital signs and baseline adverse events
- Body height and weight
  At entry: body height and weight for all patients
  During follow up: patients ≥ 18 years: body weight at every physical examination
  patients < 18 years: body height and weight at every physical examination
- GvHD evaluation (paragraph 10.3)

GvHD evaluation
See paragraph 10.3.

Toxicity / Adverse events
Toxicities / Adverse events will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 4.0 (see appendix D).

Hematology
- Hemoglobin, Leukocyte count, differential count, Platelets.

Blood chemistry
- Sodium, Potassium, Calcium, Creatinine, ASAT, ALAT, γGT, Alkaline phosphatase, Total bilirubin, Total proteins, Albumin, LDH.

Viral screening
- Quantitative CMV loads.
- EBV, AdV, other: according to institutional guidelines.
Calcineurin inhibitor trough levels
- CsA: 200-400 μg/L (using the immuno-assay), 200-300 μg/L (using the HPLC).
- Tacrolimus: 10-20 μg/L in adults, 5-15 μg/L in children.

Biopsy of involved tissue
Histology confirming GvHD of gut and/or liver should be obtained at entry if not obtained prior to start of steroid treatment. In case of gut and liver involvement, histology of either one of these tissues is considered sufficient. If biopsy of gut and/or liver has not been obtained and is not feasibly at entry for reasons of patient safety, and patients are otherwise eligible, the principal investigator should be contacted.

Histology of involved tissue at day 29 is required only in case of absence of complete response. Additional histology for immunological monitoring is required in the following cases, provided that separate patient informed consent has been obtained:
- GvHD of the skin (all stages): 2 skin biopsies at entry and at day 29 (adults only)
- sampling of involved tissue for any indication as part of standard patient care between entry and day 90: 2 biopsies.

For details on biopsy processing and transport please refer to Appendix G.

For details on the pathology review please refer to paragraph 10.5.

10.3 GVHD response evaluation
Acute GvHD response will be evaluated according to the 1994 Consensus Conference on Acute GvHD Grading Criteria (Appendix A and B). Chronic GvHD will be evaluated according to the Seattle criteria (Appendix A). The following must be scored by the treating physician and noted in the patient’s medical chart:
- Rash (% body surface)
- Liver (total bilirubin in μmol/L)
- Diarrhea (ml/day)
- Severe abdominal pain and/or ileus
- Chronic GvHD (localized or extensive) localized skin involvement and/or hepatic dysfunction due to chronic GvHD

At randomization and on day 29, GvHD clinical evaluation must be performed by two physicians and the communal result noted as such in the patient’s medical chart.
10.4 Quality of Life assessment

QoL will be assessed by means of the following questionnaires:

- **EORTC QLQ-C30 questionnaire (patient ≥ 18 years)**
  The QLQ-C30 is a multidimensional, cancer-specific quality-of-life questionnaire developed by the European Organization for Research and Treatment of Cancer (EORTC) Study Group on Quality of Life for use in international clinical trial settings. The questionnaire is designed for use with a wide range of cancer patient populations, irrespective of specific diagnosis. The QLQ-C30 includes 5 functional scales (physical, role, emotional, social and cognitive functioning), 3 symptom scales (fatigue, pain, and nausea and vomiting), a global health status/quality of life scale and a number of single items assessing additional symptoms (dyspnoea, sleep disturbance, constipation and diarrhoea) and perceived financial impact. For the majority of the QLQ-C30 items a 4-point Likert-type response scale is used. Exceptions are the items for the global quality of life scale (where a 7-point scale is used). All subscale and individual item responses are linearly converted to 0 to 100 scales. For the functional and global quality of life scales, a higher score represents a better level of functioning. For the symptom scales and items, a higher score reflects a greater degree of symptomatology.

- **FACT-BMT (patient ≥ 18 years)**
  The Functional Assessment of Cancer Therapy-Bone Marrow Transplant (FACT-BMT) Version 4 was designed to assess multi-dimensional aspects of the QoL in BMT patients (R.P. Quellon et al. BMT. 1997;19:357-368). It consists of the 27-item FACT-General (FACT-G) and the 23-item BMT Subscale (BMTS). The FACT-G assesses four primary dimensions of QoL, including physical well-being, social/family well-being, emotional well-being, and functional well-being. A five point Likert-type response scaling is used. The BMTS assesses the effects of specific BMT-related issues.

- **PEDsQL questionnaire (patient age < 18 years)**
  The PEDs-QoL measurement model is a modular approach to measuring health-related quality of life in healthy children and adolescents and those with acute and chronic health conditions. The PEDsQL questionnaire has been validated for use in multiple languages, including Dutch, German, Italian and Swedish. and has been approved for use in the evaluation of Health-related Quality of Life research in childhood survivors of cancer, including childhood survivors of GvHD treated with MSC.

- **EQ-5D questionnaire (patient age ≥ 18 years)**
  The EQ-5D is a brief, multiattribute, preference-based health status measure, developed by the EuroQol Group in order to provide a simple, generic measure of health for clinical and economic appraisal. It consists of 2 pages: the EQ-5D descriptive system, comprising 5 dimensions (mobility, self-care, usual activities, pain/discomfort and anxiety/depression) and the EQ visual analogue scale reporting the respondent’s self-rated health on a 20 cm vertical, visual analogue scale. The EQ-5D
provides a single index value for health status that can be used in the clinical and economic evaluation of health care. Currently, a pediatric version is being developed that we intend to incorporate in this study provided that validation is completed.

All patients participating in the study are expected to take part in the QoL assessment. In each participating center a QoL coordinator will be assigned who will be responsible for the QoL questionnaire collection.

The QoL coordinator will be notified by e-mail as soon as a patient is randomized at the HOVON Data Center (HDC). Patient study number, date of birth and date of randomization are mentioned in this e-mail. The baseline questionnaire will be handed to the patient by the QoL coordinator.

At the following time points the coordinator will be reminded in time to hand over the questionnaire at the correct date:
- at randomization prior to MSC treatment,
- at day 29,
- at 3, 6, 12 and 24 months during follow-up.

The QoL coordinator will collect the questionnaire from the patient and send it to HDC. If a QoL questionnaire has not been received by HOVON Data Center within 14 days of the expected date, a reminder/request will be sent to the local QoL coordinator to collect and send in the questionnaire.

The quality of life measurements will be stopped at hematological disease relapse of progression.

### 10.5 Pathology review

Following enrollment of the last study patient, the coordinating review pathologist will be notified by the HOVON Data Center by e-mail. The local trial center pathologists will receive a request to send material to the coordinating review pathologist. In this request it is mentioned that there is informed consent of the patient for review. According to the guidelines of HOVON, the name of the patient should be omitted from all correspondence, but the pathology number of the specimen, hospital number, age and gender of the patient should be provided.

2 unstained pathology slides containing 4 sections of material each as well as a copy of the corresponding pathology report are to be sent of the following histological material:
- all representative biopsies (skin, gut and/or liver) performed to confirm the diagnosis of acute GvHD
- all representative biopsies performed on day 29 to analyse the absence of complete response

Central review is performed to confirm the diagnosis of acute graft-versus-host disease grade II-IV with gut and/or liver involvement, and will be done without knowledge of patient outcome. A copy of the results of the review will be sent to the local pathologist and to the HOVON Data Center. The slides will not be returned.
All histological materials are to be sent to:
Dott.ssa Rita De Vito
Servizio Anatomia Patologica
Ospedale Pediatrico Bambino Gesù
Piazza S Onofrio.4- 00168 Roma
Tel 39 0668592740
Fax 39 0668592361

10.6 Immunological monitoring and biomarker analysis

The mechanisms by which MSC exert their in vivo functions, either through cell-to-cell contact or secretion of soluble factors or both, are still incompletely understood, and the limited data available have mainly been obtained in in vitro studies.

As an integral part of this study protocol, immunological monitoring will be performed to establish the immune reconstitution (of T, B and NK cells and subsets) and to dissect possible underlying mechanistic pathways underlying MSC efficacy. Biomarker analysis will be established to confirm the clinical evaluation of responses and to identify a laboratory signature predictive for patients responding to MSC treatment. For this purpose, blood samples will be drawn at 8 different time points (6 of which simultaneous with regular blood draws). In addition, adult patients with GvHD of the skin will be requested to donate 2 skin biopsies at entry and 2 skin biopsies at day 29 to be obtained under local anesthesia.

Furthermore, patients with acute GvHD will pre-emptively be requested to donate a blood sample prior to the start of steroid treatment. For this procedure separate informed consent will be obtained. If the patient does not enter the study, this blood sample will be destroyed. All biological samples obtained for immunological monitoring will be stripped from any identifying information and labeled with a code (trial name or number and patient study number as assigned at enrolment) prior to transport to the central laboratory at the Leiden University Medical Center.

For more detailed information on the side study see Appendix G.

11 Withdrawal of patients or premature termination of the study

11.1 Specific criteria for withdrawal of individual patients

Patients 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 patient from the study for urgent medical reasons. Specific criteria for withdrawal are:
Death

- Excessive toxicity of the investigational medicinal product
- Initiation of third line treatment for acute GvHD before day 29
- Progression/relapse of the underlying hematological malignant disorder.
- Breaking the blind before before day 29
- Major protocol violation
- No compliance of the patient
- Refusal to continue protocol treatment

Completion of protocol treatment is defined as completing treatment according to protocol until day 29.

11.2 Follow up of patients withdrawn from treatment

Patients who are withdrawn from treatment for other reasons than death will be followed as described in 10.2 for follow up.

For patients who are withdrawn from treatment because in hindsight they did not fulfil the eligibility criteria (see 8.1) at time of enrolment, data will be collected until 30 days after the last protocol treatment given. SAE information will be collected as described in 12.3.

No further information will be collected for patients who have withdrawn their consent. If a patient withdraws consent please consult HOVON Data Center.

Patients who are withdrawn from protocol treatment will receive medical care according to local practice.

11.3 Premature termination of the study

The sponsor 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);
- There is reason to conclude that it will not be possible to collect the data necessary to reach the study objectives and it is therefore not ethical to continue enrolment of more patients; for example insufficient enrolment that cannot be improved.
- The DSMB recommends to end the trial based on viable arguments other than described above.

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of the decision to terminate the study. The sponsor will provide information regarding the time lines of study termination and instructions regarding treatment and data collection of enrolled patients.
12 Safety

12.1 Definitions

Adverse event (AE)
An adverse event (AE) is any untoward medical occurrence in a patient or clinical study subject during protocol treatment. An AE does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Serious adverse event (SAE)
A serious adverse event is defined as any untoward medical occurrence that at any dose results in:

- Death
- A life-threatening event (i.e. the patient was at immediate risk of death at the time the reaction was observed)
- Hospitalization or prolongation of hospitalization
- Significant / persistent disability
- A congenital anomaly / birth defect
- Any other medically important condition (i.e. important adverse reactions that are not immediately life threatening or do not result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above, including suspected transmission of infectious agents by a medicinal product).

Note that ANY death, whether due to side effects of the treatment or due to progressive disease or due to other causes is considered as a serious adverse event.

Suspected unexpected serious adverse reaction (SUSAR)
All suspected Adverse Reactions which occur in the trial and that are both unexpected and serious. Suspected adverse reactions (AR) are those AEs of which a reasonable causal relationship to any dose administered of the investigational medicinal product and the event is suspected. Unexpected adverse reactions are adverse reactions, of which the nature, or severity, is not consistent with the applicable product information (e.g. Investigator’s Brochure for an unapproved IMP or Summary of Product Characteristics (SPC) for an authorised medicinal product).
12.2 Adverse event

12.2.1 Reporting of adverse events

Adverse events will be reported from the first study-related procedure until 30 days following the last MSC/placebo infusion or until the start of subsequent systemic therapy for the disease under study, if earlier.

Adverse events occurring after 30 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.

Adverse events related to infections, acute and/or chronic GvHD, and hematological disease relapse should be reported until 2 years of follow-up.

Adverse Events have to be reported on the Adverse Events CRF. Adverse Events will be scored according to the NCI Common Terminology Criteria for Adverse Events, version 4.0 (see appendix D).

Pre-existing conditions will be collected on the baseline concomitant diseases CRF, i.e. active (symptomatic) diseases of CTCAE grade $\geq 2$ diseases under treatment, chronic diseases and long term effects of past events as present at the time of baseline assessment.

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
- AE’s of CTCAE grade 1
- Abnormal laboratory values that have been recorded as being not clinically significant by the investigator in the source documents
- Progression of the disease under study; complications as a result of disease progression remain reportable Adverse Events

12.2.2 Follow up of adverse events

All adverse events will be followed clinically until they have been resolved, 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.

On the AE CRF only the incidence of adverse events is recorded. Any ongoing adverse event that increases in severity is to be reported as a new adverse event on the CRF. Other follow up information is not collected on the CRF.
12.3 Serious Adverse Events

12.3.1 Reporting of serious adverse events

Serious Adverse Events (SAEs) will be reported from the first study-related procedure until 30 days following the last MSC/placebo infusion or until the start of subsequent systemic therapy for the disease under study, if earlier.

Serious Adverse events occurring after 30 days should also be reported if considered at least possibly related to the investigational medicinal product by the investigator.

SAEs must be reported to the HOVON Data Center by fax within 24 hours 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 following events are not considered to be a Serious Adverse Event:

- Progression of the disease under study; complications as a result of disease progression remain reportable Serious Adverse Events
- Hospitalization for protocol therapy administration. Hospitalization or prolonged hospitalization for a complication of therapy administration will be reported as a Serious Adverse Event.
- Hospitalization for diagnostic investigations (e.g., scans, endoscopy, sampling for laboratory tests, bone marrow sampling) that are not related to an adverse event. Hospitalization or prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.
- Prolonged hospitalization for technical, practical, or social reasons, in absence of an adverse event.
- Hospitalization for a procedure that was planned prior to study participation (i.e. prior to registration or randomization). This should be recorded in the source documents. Prolonged hospitalization for a complication of such procedures remains a reportable serious adverse event.

The following adverse events will be considered as expected events that are disease/transplant related and do not require reporting as SAE:

- Progression of acute GvHD and complaints and complications thereof
- Chronic GvHD and complaints and complications thereof
- Infectious complications and complaints and complications thereof
12.3.2 Causality assessment of Serious Adverse Events

The investigator will decide whether the serious adverse event is related to trial medication, i.e. any of the products from the protocol treatment schedule. The decision will be recorded on the serious adverse event report. The assessment of causality is made by the investigator using the following:

<table>
<thead>
<tr>
<th>RELATIONSHIP</th>
<th>DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>UNRELATED</td>
<td>There is no evidence of any causal relationship</td>
</tr>
<tr>
<td>UNLIKELY</td>
<td>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).</td>
</tr>
<tr>
<td>POSSIBLE</td>
<td>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).</td>
</tr>
<tr>
<td>PROBABLE</td>
<td>There is evidence to suggest a causal relationship and the influence of other factors is unlikely.</td>
</tr>
<tr>
<td>DEFINITELY</td>
<td>There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.</td>
</tr>
<tr>
<td>NOT ASSESSABLE</td>
<td>There is insufficient or incomplete evidence to make a clinical judgment of the causal relationship.</td>
</tr>
</tbody>
</table>

12.3.3 Follow up of Serious Adverse Events

All serious adverse events will be followed clinically until they are resolved 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 information on SAE’s should be reported monthly until recovery or until a stable situation has been reached. The final outcome of the SAE should be reported on a final SAE report.

12.3.4 Processing of serious adverse event reports

The HOVON Data Center will forward all SAE reports within 24 hours of receipt to the Principal Investigator.
The HDC Safety Desk will evaluate if the SAE qualifies as a suspected unexpected serious adverse reaction (SUSAR). The IB will be used as a reference document for expectedness assessment for MSC and placebo.

The HOVON Data Center will ensure that a six-monthly line listing of all reported SAE’s is provided to the Ethics Committee(s) if this is required by national laws or regulations or by the procedures of the Ethics Committee.

12.4 Reporting Suspected Unexpected Serious Adverse Reactions

The HDC Safety Desk, on behalf of the sponsor, 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, and in accordance with any trial specific agreements between the sponsor and a co-sponsor.

Expedited reporting of SUSARs will occur no later than 15 days after the HOVON Data Center had first knowledge of the serious adverse event. For fatal or life-threatening cases this will be no later than 7 days for a preliminary report, with another 8 days for a complete report.

The manner of SUSAR reporting will be in compliance with the procedures of the Ethics Committees and Health Authorities involved.

12.5 Pregnancies

Pregnancies of a female subject or the female partner of a male subject, occurring while the subject is on protocol treatment or within 30 days following the last dose of any drug from the protocol treatment schedule, should be reported to the sponsor. Pregnancies must be reported to the HOVON Data Center by fax within 24 hours after the event was known to the investigator, using the pregnancy report form provided.

The investigator will follow the female subject until completion of the pregnancy, and must notify the sponsor of the outcome of the pregnancy within 5 days or as specified below. The investigator will provide this information as a follow-up to the initial pregnancy report. If the outcome of the pregnancy meets the criteria for classification as a SAE (i.e., spontaneous or therapeutic abortion, stillbirth, neonatal death, or congenital anomaly - including that in an aborted fetus), the investigator should follow the procedures for reporting SAEs. In the case of a live “normal” birth, the sponsor should be informed as soon as the information is available. All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days
that the investigator suspects is related to the in utero exposure to the investigational medicinal product(s) should also be reported.

The investigator is encouraged to provide outcome information of the pregnancy of the female partner of a male subject, if this information is available to the investigator and the female partner gives her permission.

12.6 Reporting of safety issues

The sponsor will promptly notify all concerned investigators, the Ethics Committee(s) and the regulatory authorities of findings that could affect adversely the safety of patients, impact the conduct of the trial, increase the risk of participation or otherwise alter the EC's approval to continue the trial. In the occurrence of such an event the sponsor and the investigators will take appropriate urgent safety measures to protect the patients against any immediate hazard. The accredited Ethics Committee will suspend the study pending further review, except insofar as suspension would jeopardize the patient's health. The local investigator will inform the patients and local ethics or review committees according to hospital policy. The sponsor will inform any other parties that are involved in the trial.

12.7 Annual safety report

The sponsor will submit once a year a safety report to the Ethics Committees and Competent Authorities of the concerned Member States. The first report is sent one year after the first approval date of the trial. The last report is sent one year after the last patient has completed protocol treatment. The content of the annual safety report will be according to the EU guidance document ‘Detailed guidance on the collection, verification and presentation of adverse reaction reports arising from clinical trials on medicinal products for human use’.

12.8 Data Safety and Monitoring Board

The DSMB will advise the Principal Investigator, co-investigators and the chair of the working group in writing about the continuation of the trial. The DSMB will review the general progress and feasibility of the trial, the quality and completeness of the data, adverse events and safety, and differences in results between the arms of a randomized trial. The DSMB will consider if there is any concern regarding the safety and well-being of trial subjects or regarding the scientific validity of the trial results. The DSMB will base her advice on the reports provided by the statistician. The DSMB is free to take into consideration external information, such as the (interim) results of other trials or literature reports.
The DSMB consists of at least three members, with at least one statistician and two physicians. Details of the DSMB constitution and tasks are documented in the trial specific DSMB charter.

The DSMB will receive at least the following reports from the trial statistician for review:
- Interim analysis report (as described in 14.3)
- Annual safety data listing the incidence of (serious) adverse events, (serious) adverse reactions and SUSARs
- Annual progress data listing the number of enrolled patients and the status of data collection

13 Endpoints

13.1 Primary endpoint

- Proportion of patients with complete or partial response to treatment of acute GvHD grade II-IV with (with gut and/or liver involvement) at day 29 (for response criteria see Appendix B).

Treatment failure is defined as:
- Failure to achieve partial or complete response at day 29.
- Initiation of third line treatment for acute GvHD prior to day 29.

13.2 Secondary endpoints

- Overall survival, defined as time from randomization until death from any cause. Patients alive at the date of last contact will be censored
- Progression-free survival, defined as time from randomization until progression or relapse of hematological malignancy or death, whichever comes first
- Duration of acute GvHD response, defined as time from response of acute GvHD until relapse of acute GvHD or death, whichever comes first
- Time from end of systemic immunosuppressive treatment for GvHD until re-initiation of systemic immunosuppression for GvHD
- Cumulative incidents of mortality not due to relapse of hematological malignancy (non-relapse mortality)
- Adverse events
- Incidence of chronic GvHD
- Quality of life
14 Statistical considerations

14.1 Patient numbers and power considerations

In order to detect an increase in the acute GvHD response rate \((CR_{GvHD} + PR_{GvHD})\) at day 29 from 40% to 65% (2-sided significance level \(\alpha = 0.05\), power \(1 - \beta = 0.80\)), 70 patients per treatment arm are required. In order to overcome dropout due to ineligibility, 150 patients will be randomized.

It should be noted that these percentages are somewhat lower that the response rates mentioned in paragraphs 5.1 and 5.3, but our response is already determined at day 29 (to allow treating physicians the option of exploring other therapies in the case of treatment failure, a potentially life-threatening situation). As such, patients with a later response are considered a failure.

Because of the clinical importance of the endpoint “overall survival”, we have performed a separate power calculation for this secondary endpoint. Assuming 70 patients per treatment arm are randomized, an increase in 1-year overall survival from 30% to 53% could be detected with a power of 80% (2-sided significance level \(\alpha = 0.05\)). However, it should be noted that the actual power may be somewhat lower as children failing second line treatment may be eligible for salvage treatment with open label MSC.

14.2 Statistical analysis

All analyses will be according the intention to treat principle, i.e. patients will be analyzed according to the treatment arm they were assigned to.

However, patients initially randomized but considered ineligible afterwards based on information that should have been available before randomization, will be excluded from all analyses.

The aim of randomization is to evaluate whether standardized second line treatment in combination with MSC infusion results in a higher proportion of patients with a complete or partial response of acute GvHD grade II-IV with gut and/or liver involvement at day 29 compared to patients treated with standardized second line treatment in combination with placebo. A patient counts as a success if he has a complete or partial response at day 29, without being a treatment failure (as defined in 13.1) at an earlier time point. All other patients will be considered a failure.
14.2.1 Efficacy analysis

The proportion of patients with a complete or partial response of acute GvHD response at day 29 will be calculated for each arm with 95% confidence intervals. The proportions will be compared between the two treatment arm using logistic regression, with adjustment for the stratification factors age (< 18 vs ≥ 18 years) and timing of GvHD-occurrence (excluding center).

The final analysis will not be performed until the acute GvHD response data for all patients have been validated.

14.2.2 Toxicity analysis

The analyses of treatment toxicity will be done primarily by tabulation of the incidence of adverse effects post-randomization with CTCAE grade 2 or more (appendix D) within 6 months after initiation of treatment for steroid-refractory acute GVHD. Specific attention will be paid to CTCAE grade 3-4 bacterial, viral, and fungal infections.

14.2.3 Additional analyses

Progression free survival (PFS) and overall survival (OS) will be estimated by the Kaplan-Meier method, and 95% confidence intervals (CIs) will be constructed. The respective hazard ratios (HRs) with 95% CIs will also be calculated. Kaplan-Meier curves will be generated to illustrate survival, and they will be compared between the 2 treatment arms using the logrank test. Competing risk analysis will be used to calculate cumulative incidences of PFS, progression/relapse and death without progression (which add up to 100% at every time point).

For each patient chronic GvHD will be evaluated, i.e. the time and grade of first onset, as well as the time until maximum grade of chronic GvHD.

14.2.4 Statistical analysis of the quality of life assessment

For this study it is hypothesized that there will be a difference between treatment arms for at least global quality of life (QoL). A mean difference of ten points between treatment arms on this scale will be considered as clinically relevant. Based on this assumption and a standard deviation of approximately 25 for this scale, the minimal effect size (difference in mean scores between treatment arms, divided by the pooled within sample estimate of the population standard deviation) is 0.40. An effect size of 0.40 is generally considered to be of medium magnitude.

In this trial, with 75 included patients per treatment arm, and using a 2-sided significance level $\alpha = 0.05$, we have 69% power to detect a 10-point difference in global QoL.
All patients with at least one follow-up QoL questionnaire will be included in the analysis. To evaluate the change in QoL over time with respect to the multi-item scales of the QLQ-C30, the repeated measures will be analyzed using mixed ANOVA models. The single items will be analyzed using (ordinal) logistic regression with random effects. The items concerning the diagnosis specific symptoms will be summarized using the unweighted sumscore. The reliability and validity of this sumscore will be established using baseline data and, when sufficient, the effect of treatment on this sumscore will be evaluated using mixed ANOVA models.

14.2.5 Statistical analysis plan

Before the final analysis, a SAP will be prepared by the trial statistician and approved by the principal investigator. It will describe in detail the analyses to be performed. Deviations from the analyses as specified in the above paragraphs will be discussed with the study coordinators and can only affect the exploratory analyses, but not the primary (confirmatory) analysis on which the sample size is based. All analyses except the primary analysis should be considered as hypothesis-generating only.

14.3 Interim analysis

Two interim analyses will be performed, based on the complete (until at least day 29) and validated data of the first 25 (1st interim analysis) and first 50 (2nd interim analysis) randomized patients per treatment arm, primarily to guard against unfavorable results in the MSC arm. A lower response rate in the MSC arm with a P-value < 0.1 (Fisher's exact test) might be a reason to recommend stopping of the trial or recommendations for modifications. A benefit in terms of response rate in the MSC arm is in general no reason to recommend early stopping of the study, unless the associated P-value is very extreme (P < 0.001, Fisher's exact test). The study will be closely and sequentially monitored before the interim analysis. Monitoring will be based on the reported SAE's, which are not subjected to data delay. The difference in the number of patients with an SAE in both arms and the difference in the number of deaths in both arms will be tested using the logrank test. It will be repeatedly tested whether those incidences in the experimental arm are higher at a significance level of 0.05. If one of both incidences is significantly higher in the MSC arm an early report will be presented to the DSMB. Results of the interim analysis will be presented confidentially only to the DSMB. Only if the DSMB recommends that the study should be stopped or modified, the results will be made public to the principal investigators for further discussion. A detailed report will be generated and presented to the DSMB. The report includes by treatment arm: the number of entered patients and at that time evaluable patients, treatment given, the number of PR\textsubscript{GVHD}, CR\textsubscript{GVHD} and failures, the incidence of side effects and infections (CTCAE grade), including SAE's and PFS and OS including causes of death.
The DSMB is free in her public recommendations to the principal investigators and her confidential recommendation to the trial statistician.

15 Registration and Randomization

15.1 Regulatory Documentation

Required regulatory and administrative documents must be provided to the HOVON Data Center before enrolment of the first patient. This will always include an Ethics Committee approval for the investigational site. The HOVON Data Center will provide each investigator with an overview of the required documents. Each investigational site will be notified when all requirements are met and enrolment can start.

15.2 Registration and Randomization

Eligible patients should be registered and randomized before start of treatment. Separate informed consent will be obtained for this pre-emptive collecting samples for immunological monitoring. Patients will only be randomized if they meet the eligibility criteria on day 5 – 12 following start of systemic steroid treatment.

Prior to randomization trial sites should contact the medical officer on call for the appropriate MSC/placebo production center to check that MSC are available (for the Netherlands: Immunohematology & Bloodtransfusion department of the LUMC +31.71.5269111, 24 hours a day). Participating trial sites that obtain their trial MSC/placebo product from another MSC-producing site should make a similar check prior to registering a patient.

Patients need to be randomized at the HOVON Data Center by one of the following options:
- Trial Online Process (TOP, https://www.hdc.hovon.nl/top). A logon to TOP can be requested at the HOVON Data Center for participants.
- By faxing the completed registration/randomization CRF +31.10.7041028 Monday through Friday, from 09:00 to 17:00 CET
- By phone +31.10.7041560 Monday through Friday, from 09:00 to 17:00 CET

The following information will be requested at registration:
- Protocol number
- Institution name
- Name of caller/responsible investigator
All eligibility criteria will be checked with a checklist. Patients will be randomized, stratified by age (<18 versus ≥18), timing of GvHD-occurrence (following HSCT or following DLI) with a minimization procedure, ensuring balance within each stratum and overall balance.

At registration each patient will be immediately be given a unique patient study number by the HOVON Data Center, either by TOP or by phone, and confirmed by fax or email. The patient study number and the result of randomization will then be communicated by the HOVON Data Center by email to the medical officer on call for the MSC/placebo production center. The result of the randomization will not be conferred to caregivers, patients, or their representatives until the end of the trial. Following randomization, the trial site may have to take additional steps to order MSC/placebo products depending on the local procedures of the associated MSC/placebo producing site. For the Netherlands, these procedures are detailed in the documents “HOVON 113 study product administration in the LUMC” and “HOVON 113 study product administration outside of the LUMC”.

Local Patient Code is a code assigned to the patient by the investigational site for local administrative purposes. The code may be up to 8 characters long (letters and numbers allowed). The code should be in compliance with privacy regulations. It should not contain identifying data, such as patient initials or the complete hospital record number. The local code will be visible in the confirmation messages sent by TOP to local participants after registration of the patient. The key to this local patient code should only be accessible by the local investigator and the local trial staff. Using or entering a local patient code is not obligatory.

15.3 Unblinding procedures

The protocol treatment will be unblinded 2 years after inclusion of the last patient. While the safety of patients should always take priority, maintenance of blinding is crucial to the integrity of a double-blind trial. Before this planned unblinding, the blind for a specific patient should only be broken when information about the patient’s protocol treatment is considered necessary to manage Serious
Adverse Events (emergency unblinding). Unblinding procedures should preferably be initiated only after consultation of the principal investigator/coordinating investigator or his/her representative. To initiate an emergency unblinding the medical officer on call for the MSC/placebo production center should be contacted (see contact details on page 3). Breaking the blind on a patient will be logged and reported by the MSC/placebo production center to the HOVON Data Center by fax within 24 hours following the unblinding procedure, using the Emergency Unblinding Form. It is considered a major protocol violation, after which the patient goes off protocol treatment (if applicable).

16 Data collection and quality assurance

16.1 Case Report Forms

Data will be collected on Case Report Forms (CRF) to document eligibility, safety and efficacy parameters, compliance to treatment schedules and parameters necessary to evaluate the study endpoints. Data collected on the CRF 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;
- Baseline concomitant diseases and 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.

Each CRF page will be identified by a trial number, and a combination of patient study number (assigned at registration) and hospital name.

The CRF will be completed on site by the local investigator or an authorized staff member. Each page must be signed by the local investigator upon completion. All CRF entries must be based on source documents.

The CRF and instructions for completing the CRF will be provided by the HOVON Data Center. The CRF pages must be made available to the HOVON Data Center at the requested time points as specified in the CRF instructions.

All data will be collected in the study database by the HOVON Data Center.
16.2 Data quality assurance

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators and appropriate study centers, review of protocol procedures with the investigator before the study, and site visits by the sponsor.
Data collected on the CRF will be verified for accuracy. If necessary, queries will be sent to the investigational site to clarify the data on the CRF. The investigator should answer data queries within the specified time line.

16.3 Monitoring

This trial is part of the HOVON Site Evaluation Visit program. Site evaluation visits will be performed for HOVON trials to review the quality of the site and not specifically the quality of a certain trial. It will enable HOVON to collect quality data and facilitate improvement of the participating sites. Data cleaning or monitoring of the performance of specific trials is not the goal of the site evaluation visits.
Site evaluation visits will be performed according to the site evaluation visit plan.
The HOVON site evaluation visit plan applies to sites in the Netherlands and Belgium only. Monitoring of the quality of trial conduct in participating sites from other countries will be organized by the coordinating investigator or co-sponsor. The frequency and content of the site visits in other countries will be at least equal to the specifications of the site evaluation visit plan, and are described in a monitoring plan provided by HOVON.
Direct access to source documentation (medical records) must be allowed for the purpose of verifying that the data recorded in the CRF are consistent with the original source data. The sponsor expects that during site visits the relevant investigational staff will be available, the source documentation will be available and a suitable environment will be provided for review of study-related documents.

16.4 Audits and inspections

The investigator will permit site-visits to carry out an audit of the study in compliance with regulatory guidelines. These audits will require access to all study records, including source documents, for inspection and comparison with the CRFs. Patient privacy must, however, be respected.
Similar auditing procedures may also be conducted by agents of any regulatory body reviewing the results of this study. The investigator should immediately notify the sponsor if they have been contacted by a regulatory agency concerning an upcoming inspection.
17 Ethics

17.1 Accredited ethics committee

An accredited Ethics Committee will approve the study protocol and any substantial amendment.

17.2 Ethical conduct of the study

The study will be conducted in accordance with the ethical principles of the Declaration of Helsinki, the ICH-GCP Guidelines, the EU Clinical Trial Directive (2001/20/EG), and applicable regulatory requirements. The local investigator is responsible for the proper conduct of the study at the study site.

17.3 Patient information and consent

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.

Since the patient should be included within 3 days after the patient becomes eligible (see chapter 8.1) there is a limited timeframe for the patient to make a decision.

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.

For children below the age of 12 years old written informed consent will be obtained from the parents or legal guardians. Children aged between 12-18 years of age will be asked to give informed written consent together with their parents’ or legal guardians’ written informed consent. Written informed
consent by both the child aged 12-18 years and the parents/legal guardians is required for enrolment in the trial. Incapacitated persons aged 12 years and older cannot be enrolled in the trial.

17.4 Benefits and risks assessment.

Acute GvHD grade II-IV with gut and/or liver involvement is a severe and potentially life-threatening complication of HSCT and DLI. The mainstay of treatment consists of corticosteroids in high dosage. In case of resistance to steroid treatment, overall survival is poor with reported mortality rates from 50% to 90%.

In this study, patients with severe steroid-refractory acute GvHD will be treated with standardized second line therapy in combination with infusion of either MSC of placebo. Based on the available phase II toxicity and feasibility data we hypothesize that early intervention with MSC in combination with standardized second line therapy will be superior in outcome and survival than standardized second line therapy alone. Documented toxicity of MSC infusions has been limited to infusion reactions. Long-term data are not yet available.

Theoretical risks include suppression of immune responses resulting in an increased rate of infections and of relapse of the original malignancy, and tumorigenesis. These potential risks, however, are balanced by the expected benefit of MSC therapy namely resolution of GvHD with its known high mortality rate both from GvHD itself and from the opportunistic infections associated with the current immunosuppressive GvHD treatment strategies.

Following HSCT patients remain under life-long surveillance. Study-related site visits, physical examinations, and drawing of blood samples will be coordinated to coincide with regular follow-up. Additional burden for the patients will consist of QoL-questionnaires, the drawing of additional blood samples, and the performing of skin biopsies.

Children may be included in this trial. Hematological malignancies are the most frequent cause of childhood cancer. Following HSCT children are at increased risk for the development of acute GvHD because of the trend towards high risk HSCT in this age group. Children are therefore a major subgroup of the patient population for which MSC treatment is being tested. MSC have been administered to children in a number of phase I/II studies with promising results and no indication of increased toxicity or adverse events in this vulnerable patient population. We therefore believe that it is both ethically justified and necessary to include children in this trial.
17.5 Trial insurance

Prior to the start of the trial, the sponsor will ensure that adequate insurance for patients is in place covering losses due to death or injury resulting from the trial, in accordance with applicable laws and regulations in each country where the trial is conducted. The sponsor will take out an insurance policy or delegate this responsibility to a national co-sponsor. Proof of insurance will be submitted to the Ethics Committee.

In addition, the sponsor will ensure that adequate insurance is in place for both investigator(s) and sponsor to cover liability pertaining to death or injury resulting from the trial.

18 Administrative aspects and publication

18.1 Handling and storage of data and documents

18.1.1 Patient confidentiality

Each patient is assigned a unique patient study number at enrolment. In trial documents the patient’s identity is coded by patient study number as assigned at enrolment. In some cases date of birth is also listed.

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 or a regulatory agency for the purpose of monitoring visits or audits and inspections.

18.1.2 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 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.

18.1.3 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.

### 18.1.4 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 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).

### 18.2 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the Ethics Committee 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 patients 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 submitted to the Ethics Committee and to the Competent Authority.

Non-substantial amendments will not be submitted, but will be recorded and filed by the sponsor.

### 18.3 Annual progress report

The sponsor will submit a summary of the progress of the trial to the accredited Ethics Committee once a year. The first report is sent one year after the first approval date of the trial. The last report is sent one year after the last patient has completed protocol treatment. Information will be provided on the date of inclusion of the first patient, numbers of patients included and numbers of patients that have completed the trial, serious adverse events/serious adverse reactions, other problems, and amendments.
18.4 End of study report

The sponsor will notify the accredited Ethics Committee and the Competent Authority of the end of the study within a period of 90 days. The end of the study is defined as 2 years after inclusion of the last patient.

In case the study is ended prematurely, the sponsor will notify the accredited Ethics Committee and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the primary endpoint analysis of the trial, the sponsor will submit an end of study report with the results of the study, including any publications/abstracts of the study, to the accredited Ethics Committee and the Competent Authority. Upon request of the accredited Ethics Committee or the Competent Authority the sponsor will submit an updated version of the end of study report within one year after the last patient’s last visit.

18.5 Publication policy

Final publication of trial results

Trial results will always be submitted for publication in a peer reviewed scientific journal regardless of the outcome of the trial – unless the trial was terminated prematurely and did not yield sufficient data for a publication.

The final publication of the trial results will be written by the Principal Investigator, the Co-investigators and the trial statistician on the basis of the statistical analysis performed by the trial statistician. A draft manuscript will be submitted for review to:

♦ All co-authors

♦ The chair of the relevant HOVON working group, who is entitled to share and discuss the manuscript with working group members

♦ An industry partner if so agreed in the contract between HOVON and company

After revision the final manuscript is submitted to the HOVON secretary for review of compliance with this policy. After approval by the HOVON board the manuscript will be sent to a peer reviewed scientific journal.
Authorship

Authors of the main manuscript will include the Principal Investigator, the Co-investigators, investigators who have included more than 5% of the evaluable patients in the trial (by order of inclusion rate), the trial statistician and the trial manager. If a substantial part of the publication is based on centrally reviewed data (e.g. cytogenetics or pathology), the central reviewer will be included as author. Others who have made a significant contribution to the trial may also be included as author, or otherwise will be included in the acknowledgement.

Authors of correlative manuscripts (e.g. results of side studies) will include the Principal Investigator, the Co-investigators, and those persons who have made a significant contribution to the published results.

The Principal Investigator should discuss and decide on the matter of authorship of the main manuscript prior to the start of the trial – with the exception of authors included on account of inclusion rate. The Principal Investigator is urged to use the maximum number of authors allowed by the journal to the full extent.

Interim and partial publications

Interim publications, abstracts or presentations of the study may include demographic data, overall results and prognostic factor analyses, results for secondary endpoints, but no comparisons between randomized treatment arms for the primary endpoint may be made publicly available before the recruitment is discontinued.

Investigators participating in the trial have a right to publish results from data they collected for the study. The Principal Investigator, the Co-investigator(s) and the trial statistician must approve any such publication, abstract or presentation based on patients included in this study. This is applicable to any individual patient or any subgroup of the trial patients. Such a publication cannot include any comparisons between randomized treatment arms nor an analysis of any of the study endpoints unless the final results of the trial have already been published.

Abstracts and presentations

Abstracts and presentations at public meetings will represent the trial as a project under HOVON affiliation. The abstract or presentation should not be represented under affiliation of the working group or a specific hospital.
Slides will be designed using the HOVON style template and any other presentation materials will show the HOVON logo.

If the trial is conducted in partnership with a co-sponsor (e.g. intergroup trial), the abstract and presentation should represent the co-sponsor contribution and slides may show the co-sponsor logo in addition to the HOVON logo.

Prior to its public use, the abstract or presentation is submitted to the HOVON secretary for review of compliance with this policy.
## Glossary of abbreviations
(in alphabetical order)

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse Event</td>
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<tr>
<td>ALL</td>
<td>Acute Lymphoid Leukemia</td>
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<tr>
<td>AML</td>
<td>Acute Myeloid Leukemia</td>
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<td>ANC</td>
<td>Absolute Neutrophil Count</td>
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<td>BM</td>
<td>Bone Marrow</td>
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<td>BMT</td>
<td>Bone Marrow Transplantation</td>
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<td>CA</td>
<td>Competent Authority</td>
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<td>CLL</td>
<td>Chronic Lymphoid Leukemia</td>
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<td>CML</td>
<td>Chronic Myeloid Leukemia</td>
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<td>CR</td>
<td>Complete Remission</td>
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<td>CRF</td>
<td>Case Report Form</td>
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<td>CTCAE</td>
<td>Common Terminology Criteria for Adverse Events</td>
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<td>DLI</td>
<td>Donor Lymphocyte Infusion</td>
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<tr>
<td>DSMB</td>
<td>Data Safety and Monitoring Board</td>
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<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
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<tr>
<td>EBMT</td>
<td>European Group for Blood and Marrow Transplantation</td>
</tr>
<tr>
<td>GCP</td>
<td>Good Clinical Practice</td>
</tr>
<tr>
<td>GI</td>
<td>Gastro-intestinal</td>
</tr>
<tr>
<td>GvHD</td>
<td>Graft-versus-Host Disease</td>
</tr>
<tr>
<td>GvL</td>
<td>Graft-versus-Leukemia</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HL</td>
<td>Hodgkin’s Lymphoma</td>
</tr>
<tr>
<td>HLA</td>
<td>Human Leukocyte histocompatibility Antigen</td>
</tr>
<tr>
<td>HOVON</td>
<td>Dutch-Belgian Hematology-Oncology Cooperative Group</td>
</tr>
<tr>
<td>HSCT</td>
<td>Hematopoietic Stem Cell Transplantation</td>
</tr>
<tr>
<td>IMP</td>
<td>Investigational Medicinal Product</td>
</tr>
<tr>
<td>ITT</td>
<td>Intention To Treat</td>
</tr>
<tr>
<td>IU</td>
<td>International Units</td>
</tr>
<tr>
<td>LDH</td>
<td>Lactate Dehydrogenase</td>
</tr>
<tr>
<td>METC</td>
<td>Medical Ethical Review Committee</td>
</tr>
<tr>
<td>MSC</td>
<td>Mesenchymal Stromal Cell</td>
</tr>
<tr>
<td>MM</td>
<td>Multiple Myeloma</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>N2</td>
<td>Nitrogen</td>
</tr>
<tr>
<td>NaCl</td>
<td>Sodium Chloride</td>
</tr>
<tr>
<td>NCI</td>
<td>National Cancer Institute</td>
</tr>
<tr>
<td>NHL</td>
<td>Non-hodgkin's lymphoma</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>OS</td>
<td>Overall Survival</td>
</tr>
<tr>
<td>PB</td>
<td>Peripheral Blood</td>
</tr>
<tr>
<td>PD</td>
<td>Progressive Disease</td>
</tr>
<tr>
<td>PFS</td>
<td>Progression Free Survival</td>
</tr>
<tr>
<td>PO</td>
<td>Per Os</td>
</tr>
<tr>
<td>PR</td>
<td>Partial Response</td>
</tr>
<tr>
<td>QoL</td>
<td>Quality of Life</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious Adverse Event</td>
</tr>
<tr>
<td>SC</td>
<td>Subcutaneous</td>
</tr>
<tr>
<td>SD</td>
<td>Stable Disease</td>
</tr>
<tr>
<td>SOP</td>
<td>Standard Operating Procedure</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected Unexpected Serious Adverse Reaction</td>
</tr>
<tr>
<td>ULN</td>
<td>Upper Limit of Normal</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>WMO</td>
<td>Wet Medisch-Wetenschappelijk Onderzoek met mensen</td>
</tr>
</tbody>
</table>
19 References


84. http://investor.osiris.com/releases.cfm


A. Criteria for diagnosis, staging and grading of GvHD

Diagnosis:
Acute and chronic GvHD is defined according to the proposal of the National Institutes of Health (NIH) Consensus Conference, which recognizes 2 categories of GvHD:
1. Acute GVHD (absence of features consistent with chronic GVHD), which may present as:
   - classic acute GvHD (before day 100 following HSCT or DLI)
   - persistent, recurrent, or late acute GvHD (after day 100, often upon withdrawal of immunosuppression), no features consistent with chronic GvHD
2. Chronic GvHD, which may present as:
   - classic chronic GvHD (no signs of acute GvHD)
   - an overlap syndrome, in which features of both acute and chronic GvHD are present


Staging of acute GvHD

<table>
<thead>
<tr>
<th>Stage</th>
<th>Skin</th>
<th>Liver</th>
<th>Intestinal Tract</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rash (% body surface)</td>
<td>Total bilirubin (μmol/L)</td>
<td>Diarrhea (ml/day)</td>
</tr>
<tr>
<td>1</td>
<td>&lt; 25</td>
<td>34-50</td>
<td>500-1000 or persistent nausea without diarrhea *</td>
</tr>
<tr>
<td>2</td>
<td>25-50</td>
<td>50-102</td>
<td>1000-1500</td>
</tr>
<tr>
<td>3</td>
<td>&gt; 50</td>
<td>102-255</td>
<td>&gt; 1500</td>
</tr>
<tr>
<td>4</td>
<td>Generalized erythroderma with bullous formation</td>
<td>&gt; 255</td>
<td>3. + severe abdominal pain / ileus</td>
</tr>
</tbody>
</table>

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

Grade

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Skin: stage 1-2 and Liver: stage 0 and Gut: stage 0</td>
</tr>
<tr>
<td>II</td>
<td>Skin: stage 3 or Liver: stage 1 or Gut: stage 1</td>
</tr>
<tr>
<td>III</td>
<td>Liver: stage 2-3 or Gut stage: 2-4</td>
</tr>
<tr>
<td>IV</td>
<td>Skin or Liver: stage 4</td>
</tr>
</tbody>
</table>
## Staging of chronic GvHD

### Seattle criteria for limited and extensive chronic GvHD

<table>
<thead>
<tr>
<th>Limited</th>
<th>Either or both:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- localized skin involvement</td>
</tr>
<tr>
<td></td>
<td>- hepatic dysfunction due to chronic GvHD</td>
</tr>
</tbody>
</table>

### Extensive

<table>
<thead>
<tr>
<th>Either:</th>
</tr>
</thead>
<tbody>
<tr>
<td>- generalized skin involvement</td>
</tr>
<tr>
<td>- localized skin involvement and/or hepatic dysfunction due to chronic GvHD, plus</td>
</tr>
<tr>
<td>any of the following:</td>
</tr>
<tr>
<td>- liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis</td>
</tr>
<tr>
<td>- involvement of eye (as demonstrated by Schirmer's test)</td>
</tr>
<tr>
<td>- involvement of salivary glands or oral mucosa as proven by labial biopsy</td>
</tr>
<tr>
<td>- involvement of any other target organ</td>
</tr>
</tbody>
</table>
B. Acute GvHD response criteria

Complete response: the return of acute GvHD to grade 0
Partial response: at least 1 grade improvement in GvHD
Mixed response: at least 1 stage improvement of at least 1 organ, with at least 1 stage worsening in at least 1 other organ
Stable disease: no significant change in any organ system
Progressive disease: at least 1 grade progression of GvHD
C. Performance score

Karnofsky performance score (patients ≥ 16 years):
- 100% – normal, no complaints, no signs of disease
- 90% – capable of normal activity, few symptoms or signs of disease
- 80% – normal activity with some difficulty, some symptoms or signs
- 70% – caring for self, not capable of normal activity or work
- 60% – requiring some help, can take care of most personal requirements
- 50% – requires help often, requires frequent medical care
- 40% – disabled, requires special care and help
- 30% – severely disabled, hospital admission indicated but no risk of death
- 20% – very ill, urgently requiring admission, requires supportive measures or treatment
- 10% – moribund, rapidly progressive fatal disease processes
- 0% – death

Lansky performance score (patients < 16 years):
- 100% – fully active, normal
- 90% – minor restrictions in strenuous physical activity
- 80% – active, but tired more quickly
- 70% – greater restriction of play and less time spent in play activity
- 60% – up and around, but active play minimal; keeps busy by being involved in quieter activities
- 50% – lying around much of the day, but gets dressed; no active playing participates in all quiet play and activities
- 40% – mainly in bed; participates in quiet activities
- 30% – bedbound; needing assistance even for quiet play
- 20% – sleeping often; play entirely limited to very passive activities
- 10% – doesn't play; does not get out of bed
- 0% – unresponsive
D. Common Terminology Criteria for Adverse Events

The grading of adverse events will be done using the NCI Common Terminology Criteria for Adverse Events, CTCAE version 4.0. A complete document may be downloaded from the HOVON website:

http://www.hovon.nl (under Trials > General information about studies)
E. MSC Infusion protocol

The infusion procedure is to be performed according to local transfusion procedures for DMSO containing cellular products. The patient will be informed by the nurse-on-duty about the procedure and possible side-effects of the investigational medicinal product, particularly: fever and chills, allergic reaction (DMSO), olfactory and taste sensations (DMSO). During the procedure a qualified physician will be available on call.

Specifically:
- The transit time between thawing and infusion of the investigational medicinal product should be as short as possible.
- The investigational medicinal product is to be infused directly, not using a pump system.
- The peripherous canule should be checked for functionality prior to infusion.
- An emergency set should be available at the bedside.
- Blood pressure, pulse frequency and temperature should be measured prior to infusion, every 15 minutes during transfusion, and 1 hour after transfusion.
- Patient identity and the investigational medicinal product ID are to be checked by at least 2 qualified nurses.
- In case of allergic reaction or shock, the infusion should be stopped directly and the physician on call should be consulted.
- The patient may be discharged 2 hours after the infusion in case of stable controls.

The overpouch containing the bag with the investigational medicinal product may not be opened before, during, or after the infusion procedure. Following the infusion procedure the overpouch containing the bag with the used investigational medicinal product must be returned to the stem cell laboratory along with a blood product infusion report form filled out according to local regulations.
F. MSC preparation protocol

The preparation of MSC is detailed in the IMPD.
G. Monitoring of clinical and laboratory parameters to document diagnosis, severity, progression and response to treatment of acute GvHD

The aims of this study are to investigate the applicability of soluble and/or cellular markers as a sensitive and specific tool to:

1. Predict the efficacy of steroid and MSC treatment of acute GVHD
2. Monitor “online” the effect of treatment next to clinical response criteria

### Monitoring schedule

<table>
<thead>
<tr>
<th>Day of study</th>
<th>Time point / period</th>
<th>Clinical evaluation(^1)</th>
<th>Blood counts</th>
<th>Soluble biomarkers (plasma)(^2)</th>
<th>Monitoring of cells (PBMC)(^2)</th>
<th>Biopsy(^2,3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior to study entry (Day ≥ -5)(^4)</td>
<td>At day of (but prior to) start of systemic steroids for acute GvHD</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X(^1)</td>
</tr>
<tr>
<td>Day 1</td>
<td>At day of (but prior to) 1(^{st}) infusion of MSC/placebo</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Day 2</td>
<td>At day following 1(^{st}) infusion of MSC/placebo</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Day 8</td>
<td>At day of (but prior to) 2(^{nd}) infusion of MSC/placebo</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Day 9</td>
<td>At day following 2(^{nd}) infusion of MSC/placebo</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Day 15</td>
<td>7 days following 2(^{nd}) infusion of MSC/placebo</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Day 22</td>
<td>14 days following 2(^{nd}) infusion of MSC/placebo</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>-</td>
</tr>
<tr>
<td>Day 29</td>
<td>End of treatment protocol</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

\(^1\) part of routine patient evaluation, see paragraph 10.3 of study protocol  
\(^2\) specifically required for immunological monitoring and biomarker analysis  
\(^3\) of involved tissue; skin: at entry and at day 29; other involved tissue: between entry and day 90 (whenever tissue samples are obtained as part of standard care); separate informed consent for the taking of biopsies must be obtained prior to study entry  
\(^4\) depending on the response to first line therapy, this time point will be day –5 to day –8 or day –10 to day –13 prior to randomization,
Required samples per timepoint for adults age ≥ 18 years:

- **Soluble Biomarkers**: 10 mL EDTA blood (for plasma); to be stored in 4 aliquots at -80°C (preferentially) or at -20°C

- **Monitoring cells**: ≥ 20 mL EDTA blood
  - for FACS analysis: 1 vial of 5-10 x 10^6 PBMC; to be stored in liquid N2
  - for future usage: 2 vials of 5 x 10^6 PBMC each; to be stored in liquid N2

- **Biopsies** skin: two 4 mm punch biopsies at each of the two time points
  - one biopsy is snap frozen, to be stored in liquid N2
  - one biopsy is formalin fixated and paraffin embedded, to be stored at room temperature

  additional involved tissue, if available (see paragraph 10.2): two biopsies per sampling
  - one biopsy is snap frozen, to be stored in liquid N2
  - one biopsy is formalin fixated and paraffin embedded, to be stored at room temperature

Required samples per timepoint for children age <18 years:

If bodyweight over 20 kg, Hb at least 6.0 mmol/L or 9.7 g/dL, and no other contra-indications for biomarker study blood sampling as determined by the treating paediatrician:

- **Soluble Biomarkers**: 10 mL EDTA blood (for plasma); to be stored in 4 aliquots at -80°C (preferentially) or at -20°C

- **Monitoring cells**: ≥ 10 mL EDTA blood
  - for FACS analysis: 1 vial of 5 x 10^6 PBMC; to be stored in liquid N2
  - for future usage: 1 vial of 5 x 10^6 PBMC each; to be stored in liquid N2

If bodyweight 10-20 kg, Hb at least 6.0 mmol/L or 9.7 g/dL, and no other contra-indications for biomarker study blood sampling as determined by the treating paediatrician:

- **Soluble Biomarkers**: 10 mL EDTA blood (for plasma); to be stored in 4 aliquots at -80°C (preferentially) or at -20°C

If bodyweight < 10 kg, Hb < 6.0 mmol/L or 9.7 g/dL, or other contra-indications for biomarker study blood sampling as determined by the treating paediatrician:

- **No sampling for biomarker study**
All children:
If samples of involved tissue (skin, gut, or liver) are taken for any indication as part of standard patient care between entry and day 90 and if there are no contra-indications for additional tissue sampling as determined by the treating paediatrician and if informed consent for additional sampling has been obtained:

- Two biopsies: - one biopsy is snap frozen, to be stored in liquid N₂
  - one biopsy is formalin fixated and paraffin embedded, to be stored at room temperature

Because monitoring of biomarkers and cells are scheduled at the same day, in practice no withdrawal of additional EDTA blood is required for saving of plasma.

Processing and transport:
All samples are to be processed by the local institute.
Vials and biopsies should be labelled as indicated in the table and shipped batchwise after reaching the end of the treatment protocol (day 29) by courier service under the conditions indicated in the table below.

<table>
<thead>
<tr>
<th>Material</th>
<th>Label: Patient Study number</th>
<th>Label: Content</th>
<th>Label: Sampling Date</th>
<th>Label: Number of cells</th>
<th>Conditions for transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma vials</td>
<td>Yes</td>
<td>Plasma</td>
<td>Yes</td>
<td>No</td>
<td>On dry ice</td>
</tr>
<tr>
<td>PBMC vials</td>
<td>Yes</td>
<td>PBMC</td>
<td>Yes</td>
<td>Indicate number e.g. 6 x 10⁶</td>
<td>On dry ice</td>
</tr>
<tr>
<td>Snap frozen biopsies</td>
<td>Yes</td>
<td>Involved tissue</td>
<td>Yes</td>
<td>No</td>
<td>On dry ice</td>
</tr>
<tr>
<td>Paraffin embedded biopsies</td>
<td>Yes</td>
<td>Involved tissue</td>
<td>Yes</td>
<td>No</td>
<td>Room temperature</td>
</tr>
</tbody>
</table>

Address for shipment: Immunological Laboratory (P3-P)
Department of Pediatrics
Leiden University Medical Center
Albinusdreef 2
2333 ZA Leiden, The Netherlands
Central laboratory analysis (LUMC): 

**Soluble biomarkers in plasma:**

- **Tissue damage (skin, gut, liver):**
  - Skin: Elafin (Paczesny et al., 2010)
  - Gut, liver: Cytokeratin-18 (Luft et al., 2007)
  - Gut: REG-3α (Ferrara et al., 2011; Harris et al., 2012)

- **Endothelial damage/inflammation:**
  - VEGF (Lunn et al., 2005)
  - ANG2/VEGF (Luft et al., 2011)

- **“Diagnostic panel” of biomarkers aGVHD:**
  - sIL-2Rα, sTNF-R1, IL-8, HGF (Paczesny et al., 2009)
  - Panel + Elafin and REG-3α (Levine et al., 2012)
  - sIL-2Rα (von Bahr et al., 2011)

- **Mediators immune modulation by MSC:**
  - sHLA-G5 (Le Maux et al., 2008; von Bahr et al., 2011)
  - Galectin-1 (Gieseke et al., 2010)

- **Markers for inflammatory/tolerogenic state:**
  - IL-6, IL-10 (Fujii et al., 2006), (TGFβ)

**Monitoring of cells:**

- **T cells:**
  - CD4+ and CD8+ T-cell subsets including differentiation stages:
    - CCR7+/CD45RA+ (N), CCR7+/CD45RA- (CM),
    - CCR7/CD45RA- (EM), CCR7-/CD45RA+ (EMRA)
    (eventually combined with expression of CLA and α4β7, Engelhardt et al., 2010)
  - Th17: CD3+/CD4+/IL-17/IFN-γ/IL-23R+ after PMA/ionomycin stimulation
    (Dander et al., 2009; Ratajczak et al., 2010)

- **NK cells:**
  - CD3-/CD56dim/CD16+ and CD3-/CD56bright/CD16dim

- **B cells:**
  - CD19+/CD20+/CD27- (N) and CD19+/CD20+/CD27+ (M)
Biopsies:
- Investigation: - Hematoxylin/eosin (HE) staining;
  - Combined staining for: CD3, CD4, CD8 (T-cell infiltrate);
    CD3, CD1a (DC), CD14 and CD163 (macrophages) (Nishiwaki et al., 2009);
    CD3, CD4, Foxp3 (Treg) and CD3, CD4, CCR6 (Th17).

References:
- von Bahr et al.  Biol Blood Marrow Transplant. 2011; adv. online publ., 1-8
- Engelhardt et al.  Bone Marrow Transplant. 2010; adv. online publ., 1-7.
H. Definition cause of death

**Primary Disease Recurrence**

- **Yes**
  - If death is due to *relapse* or *persistent disease*, this will be the primary cause of death.
  - Recurrent disease was only discovered by autopsy → Report relapse as a contributing cause of death.

- **No**
  - **Non-Engraftment**
    - **Yes**
      - **Graft failure** is the primary cause of death if any of the following 3 conditions is present.
        - **Condition 1**
          - Primary graft failure: ANC <500/mm³ and bone marrow examination, if performed, with <5% cellularity during first 28 days.
        - **Condition 2**
          - Must also list a secondary cause of death
        - **Condition 3**
          - *Autologous recovery*

  - **No**
    - **GVHD**
      - **Yes**
        - Acute GVHD is the primary cause of death if patient was on treatment (not prophylaxis) for GVHD even if clinical GVHD not evident at death
      - Prior to day +28
      - For patients with acute GVHD prior to day +28 and treated systemically, who also experience primary graft failure, acute GVHD will be the primary cause of death.
      - If there is an intervention and patient gets GVHD, then the reason for the intervention is the primary cause of death.

- **No**
  - **Chronic GVHD** is the primary cause of death if patient was receiving treatment for chronic GVHD even if chronic GVHD not evident at death.
Appendix H

Infection

Yes
- Protozoal
- Fungal
- Bacterial
- Viral

No

Organ Failure

Yes
- Report any organ failure that is not due to GVHD or infection. Multi-organ failure is defined as failure of more than 1 organ system. The hierarchy for single organ failures:
  1st - Secondary graft failure
  2nd - Other, multi-organ failure
  3rd - Pulmonary
  4th - Cardiac
  5th - Liver
  6th - Renal
  7th - Central nervous system

No

Secondary Malignancies

Yes
- Report secondary malignancies, but not EBV lymphoma

No

Hemorrhage

Yes
- Report hemorrhage when there is excessive bleeding, typically from the gastrointestinal tract or a ruptured blood vessel, not the central nervous system

No

Accidental Death

Yes
- Report accidental death for accidents that are unrelated to the medical treatment of the patient. These include motor vehicle accidents, falls, drownings, natural disasters, etc.

No

Other

Yes
- Report other when all other categories above do not describe the primary or contributing cause of death for such instances as suicide, heart attack, cardiac arrest, ventricular tachycardia, malignant arrhythmia, cardiopulmonary arrest, sudden death, and death from progressive nonmalignant disease (e.g., a preexisting or recurrent metabolic disorder, such as Harter Syndrome).

Reference: