A phase I/II feasibility study of the combination of panobinostat and decitabine prior to donor lymphocyte infusion in recipients of allogeneic stem cell transplantation with poor and very poor-risk AML

PROTOCOL

Principal Investigator	**:**	J.J. Cornelissen
Sponsor	**:**	HOVON
EudraCT number	**:**	2012-003344-74
Final protocol (version 01)	**:**	13 JULY 2012
Version 02	**:**	23 MAY 2013
Version 03	**:**	08 OCT 2013
Version 04	**:**	16 DEC 2013
Version 05	**:**	14 JAN 2014
Version 06	**:**	20 MAY 2014

PRINCIPAL INVESTIGATOR SIGNATURE PAGE

Signature of Principal Investigator

11-06-2014
Date

M.J. Cornelissen
Printed Name of Principal Investigator

LOCAL INVESTIGATOR SIGNATURE PAGE

Local site name: _____

_____ _____

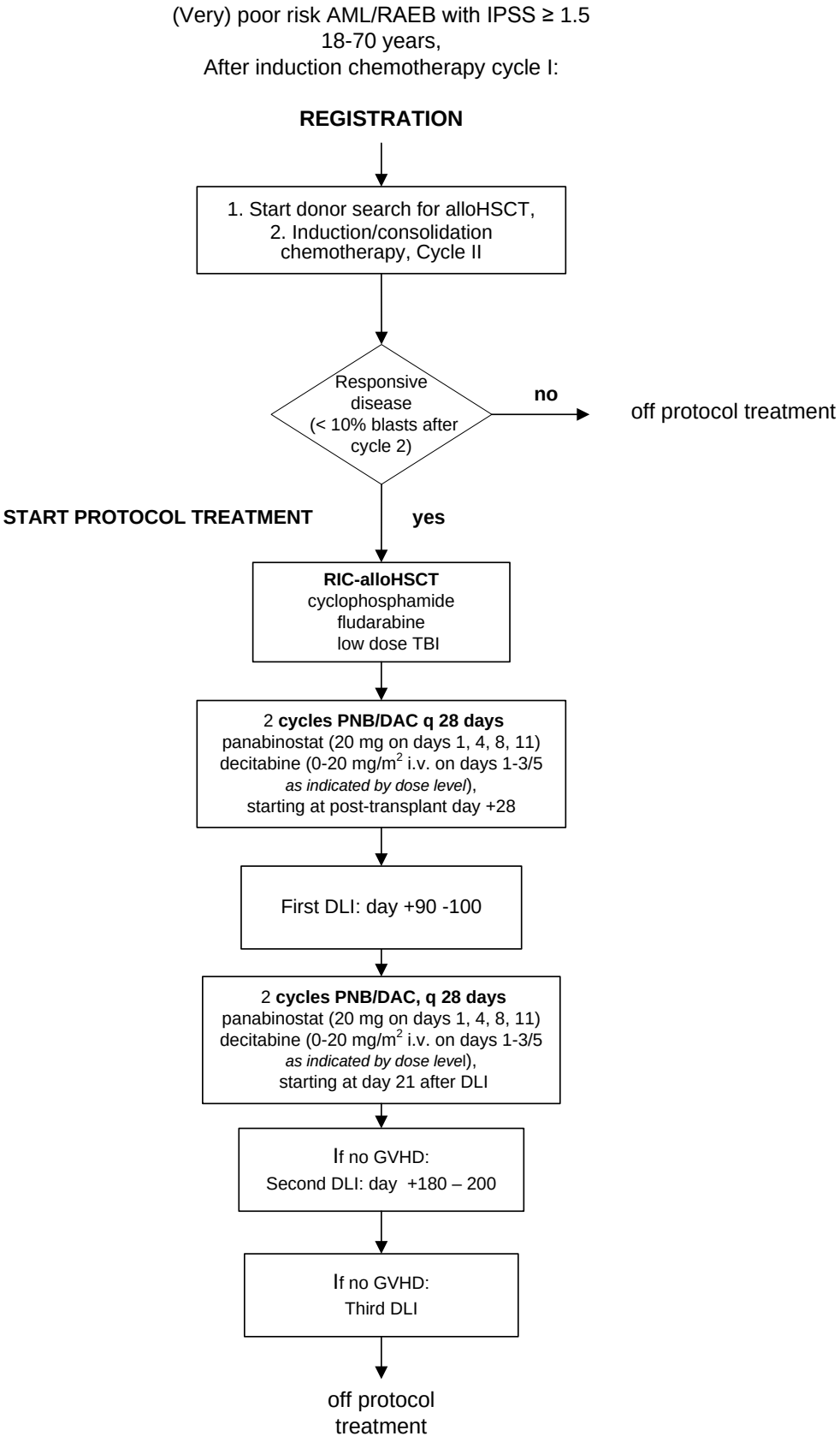
Signature of Local Investigator Date

_____

Printed Name of Local Investigator

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.

1 Scheme of study



2 Table of contents

Page

3 Synopsis

Rationale

This study will explore the feasibility of post-transplant panobinostat combined with decitabine after reduced intensity conditioning (RIC) alloHSCT in patients with (very) poor-risk AMLor RAEB with IPSS ≥ 1.5 (AML/RAEB).

While recent studies showed that the allogeneic graft-versus-leukemia (GVL) effect is clearly operational in (very) poor-risk AML, relapse rates after alloHSCT in those patients are still unacceptably high, with no curative options left. Based on recent experience by others exploring the combination of panobinostat (PNB) and decitabine (DAC) in AML patients and by different groups exploring post-transplant chemotherapy including panobinostat, we here propose to study the combination of panobinostat and decitabine after alloHSCT to be followed by DLI to optimally profit from the allogeneic GVL-effect. Feasibility in this study will be defined by the completion of protocol treatment up to eligibility for a first dose of DLI in at least 70% of patients starting protocol treatment, without dose limiting toxicity up to that point of time.

Main study objectives

Primary objective:

Part I

To asses the safety and feasibility of post-transplant panobinostat combined with decitabine to a regimen of T-cell replete RIC alloHSCT and DLI and select the dose level for part II of the study

Part II

Assess the feasibility and efficacy of post-transplant panobinostat combined with decitabine to a regimen of T-cell replete RIC alloHSCT and DLI in patients with (very) poor-risk AML

	Secondary objectives: Assess efficacy in terms of complete remission rate, overall and progression free survival. Assess toxicity.
Study design	Multicenter, prospective phase I/II trial
Patient population	Patients aged 18-70 years (inclusive), with (very) poor-risk AML or RAEB with IPSS ≥ 1.5
Intervention	Panobinostat combined with decitabine in the setting of RIC-alloHSCT The setting, framework of RIC-alloHSCT is detailed as follows: - T cell replete RIC alloHSCT with a short-course post-transplant GvHD prophylaxis consisting of high-dose cyclophosphamide and short-term ciclosporin, followed by - 2 cycles of panobinostat and decitabine (PNB/DAC), followed by - DLI at 3 months after alloHSCT, followed by - another 2 cycles of PNB/DAC, followed by - a second DLI (and third DLI), if no GvHD has developed.
Duration of treatment	Patients will be treated according to protocol until 3-12 months post alloHSCT. Subsequently patients will be followed until 5 years after registration.
Target number of patients	100 patients registered of which approximately 60 will start protocol treatment.
Expected duration of accrual	2 years
Part I Primary endpoint	Feasibility of protocol treatment as defined by the number of DLTs during the first cycle

Part II Primary endpoint	Feasibility as determined by percentage of patients eligible for first DLI according to protocol without suffering from severe toxicity.
Benefit and nature and extent of the burden and risks associated with participation	Although alloHSCT is standard care in (very) poor-risk AML/RAEB, the incidence of relapse after alloHSCT is high, leaving patients without curative options. In this protocol post-transplant PNB/DAC is evaluated. The aim is to reduce the incidence of early GvHD, by means of intensified GvHD-prophylaxis by high dose cyclophosphamide and to prevent early relapse prior to DLI by PNB/DAC. It is hypothesized that the implementation of delayed DLI-immunotherapy following epigenetic therapy results in optimal allogeneic Graft versus Leukemia effect, to improve outcome and decrease the relapse incidence. The risks associated with this procedure are opportunistic infections associated with neutropenia and lymphopenia, that may occur after PNB/DAC, as compared to standard alloHSCT.
Planned interim analysis and DSMB (if applicable)	All interim analysis reports will be sent to the DSMB. Interim analyses are performed during: - Part I: if the decision rules imply dose escalation, stopping, or continuing as part II - Part II: after 25 patients are evaluable up to DLI. The DSMB will advise the investigators on (dis)continuation of the study.

4 Investigators and study administrative structure

Responsibility	Name	Affiliation/Address
Sponsor	HOVON	VU University Medical Center, P.O.Box 7057 1007 MB Amsterdam tel: +31 20 4443797
Co-sponsor	*<if applicable>*	
Representative of Sponsor (HOVON)	P.C. Huijgens, chairman P. Sonneveld, treasurer	VU University Medical Center Amsterdam Erasmus MC Rotterdam
Principal Investigator	J.J. Cornelissen	Erasmus MC Rotterdam
Co-investigators	G.J. Ossenkoppele E. Meijer M. Jongen-Lavrencic	VU University Medical Center, Amsterdam VU University Medical Center, Amsterdam Erasmus MC Rotterdam
Coordinating investigator	*Belgium* D. Breems *Sweden* G. Juliusson *Lithuania* L. Griškevičius	 Hospital Network Antwerpen <*Center*> Stockholm <*Center*> Vilius
HOVON stem cell transplantation working group chair	J.J. Cornelissen	Erasmus MC Rotterdam
Writing Committee	G. Huls D. Breems P. von dem Borne F. Falkenburg C. Huisman M. Schaap H. Schouten J. Passweg J. Maertens M. Manz E. Vellenga G. Juliusson L. Griškevičius (Laimonas.Griskevicius@santa.lt) B. Lowenberg	Radboud University Nijmegen Medical Center, Nijmegen Hospital Network Antwerpen Leiden University Medical Center, Leiden Leiden University Medical Center, Leiden Academic Medical Center, Amsterdam Radboud University Nijmegen Medical Center, Nijmegen Maastricht University Medical Center, Maastricht University Hospital, Basel University Hospital Gasthuisberg, Leuven University Hospital, Zürich University Medical Center, Groningen Stockholm, Sweden Vilius, Lithuania Erasmus MC Rotterdam
Statistician	Y. van Norden	Erasmus MC Rotterdam

Registration	HOVON Data Center	Erasmus MC – Clinical Trial Center P.O.Box 2040 3000 CA ROTTERDAM The Netherlands https://www.hdc.hovon.nl/top tel. +31.10.7041560 fax +31.10.7041028
Monitoring	HOVON Data Center	Erasmus MC – Clinical Trial Center
Central Pharmacy	Erasmus MC	
Trial management	HOVON Data Center	Erasmus MC – Clinical Trial Center
Data management	HOVON Data Center	Erasmus MC – Cancer Trial Center
Serious Adverse Events (SAEs) notification	HOVON Data Center	Erasmus MC – Clinical Trial Center

5 Introduction and rationale

5.1 AlloHSCT in poor-risk AML

Allogeneic HSCT is generally advocated as the treatment of choice to consolidate remission in intermediate and (very) poor-risk AML in first complete remission up to 60 years of age [1, 2]. Meta-analysis of larger numbers of AML patients have shown that sibling donor availability confers a significant survival advantage of approximately 10-15% and a consistent relative reduction of relapse by approximately 0.5 (hazard ratio) as compared to conventional chemotherapy or autologous transplantation[2, 3]. The advantage of sibling donor transplants currently extends to AML recipients of well-matched unrelated donor transplants with more or less equal outcome. Therefore unrelated donors are applied with increasing frequency [4].

The last decade, new cytogenetic and molecular markers have been identified that specifically relate to poor or very poor-risk AML. The latter categories include the so-called monosomal karyotype (MK) leukemia's and those with high expression of EVI-1 [5]. Recent studies suggested that the beneficial effect of alloHSCT also applies to those categories of AML [6, 7], although alloHSCT in these newer subsets of AML is still associated with a considerable risk of relapse. Apart from cytogenetic and molecular prognostic markers, a number of other variables may offer additional prognostic information. Such variables include time to CR, number of blasts early after induction and quantified minimal residual disease after induction or consolidation. Different groups have shown that quantified levels of MRD after e.g. consolidation significantly relate to outcome and risk of relapse in first CR patients.

Patients who fail to achieve remission after one or two courses of chemotherapy usually have a dismal prognosis. The CIBMTR recently reported outcome of a large series of patients with relapse leukemia or with primary induction failure [8] and identified subgroup of AML patients with less than 3 risk factors, for whom transplantation could still be a reasonable treatment option. Therefore, patients failing induction chemotherapy may still be considered candidates for an allogeneic transplant. The urge to consider a possible transplant relatively fast is stressed by recent reports showing the importance of the number of induction courses. Schmid et al. reported relatively favourable results in patients with primary refractory AML using alloHSCT using a reduced intensity preparative regimen, preceded by a course of chemotherapy including potent anti-leukemic drugs and not awaiting hematological recovery [8]. This observation strongly suggests that early timing of alloHSCT is crucial.

Despite being more efficacious than consolidation chemotherapy, the risk of relapse in poor-risk AML is still considerable and may estimate between 30 and 60%. It suggests that, although clearly operational, the graft versus leukemia (GVL) effect has not been exploited fully. While it underscores the importance of allogeneic HSCT in poor-risk AML, improvement is eagerly awaited. Further improvement may be pursued by avoiding delay and immediate application of allogeneic HSCT. Proceeding to allogeneic HSCT may even be considered before full peripheral hematopoietic

recovery has been obtained, as pioneered by Schmid et al [9]. In addition, the continued application of new agents such as azacitidine (Vidaza®) after allogeneic HSCT [10] may offer a new approach to facilitate ongoing GVL, while inhibiting imminent relapse after transplantation.
In order to optimize at one hand disease control and, at the other hand, optimize the immunotherapeutic GVL-effect, a recent study set out to explore the combined use of azacitidine and valproic acid after allogeneic HSCT with the aim to restore epigenetic deregulation and facilitate allogeneic immunotherapy [11]. Epigenetic deregulation is a key feature in the pathophysiology of AML, resulting in aberrant transcription of genes involved in cell growth, proliferation, differentiation and apoptosis. Histone acetylation is a major epigenetic mechanism for the regulation of gene expression and is maintained by the activities of histone deacetylases (HDACs) and histone acetyltransferases (HATs) (13). Given the epigenetic deregulation observed in AML pathogenesis, agents such as histone deacetylase inhibitors (HDACi) are in clinical development for AML treatment. Attempts to develop more potent HDAC inhibitors led to the development of a novel class of cinnamic acid hydroxamate compounds with panobinostat (LBH589) as the most prominent example of this group of agents. Panobinostat has shown strong preclinical activity in several preclinical cancer models [12] [13] and is currently in clinical trials for a number of hematologic malignancies, including lymphomas, leukemias and myelodysplastic syndromes (see below). Decitabine is already approved as a hypomethylating agent for the treatment of high risk MDS and elderly AML. It offers an overall survival advantage as compared to conventional care regimens (see section 5.3).

5.2 Reduced intensity conditioning alloHSCT

Non-myeloablative or reduced intensity conditioning (RIC) regimens have been developed in order to reduce non-relapse mortality (NRM) in elderly or medically less fit patients. Several studies have indeed shown that the morbidity and mortality following RIC alloHSCT are less than after myeloablative (MA) conditioning, whereas encouraging GVL effects are exerted [14-16]. Most comparative studies reported were performed retrospectively and concerned patients with AML/MDS in 1st CR, 2nd CR, or with advanced disease. The majority of these studies indeed showed a lower NRM after RIC conditioning as compared to MA conditioning, especially as patients assigned to RIC regimens were generally older and had higher comorbidity scores. During the prospective AMLHD 98A study [17], a growing number of patients received various dose-reduced conditioning regimens. Although not randomized, that study prospectively suggested equivalent results in patients receiving dose reduced and myeloablative conditiong regimens in terms of relapse, NRM, and survival. While several retrospective studies have suggested a somewhat higher relapse rate in recipients of RIC alloHSCT ([18, 19], two very recent studies suggested that relapse may not differ in intensively pretreated AML CR1 patients, who subsequently proceeded to either RIC alloHSCT or MA alloHSCT[20, 21] A prospective comparison of older AML patients by sibling donor availability suggested improved disease free survival for patients with a donor [22]. A recent large retrospective

studies in older cohorts of AML patients also strongly suggested improved outcome in recipients of RIC alloHSCT as compared to conventional chemotherapy [23]. Collectively, these studies strongly suggest that the beneficial effect of alloHSCT largely depends on the immunotherapeutic GVL effect and less on the intensity of the conditioning regimen in AML patients, who attained first CR upon intensive chemotherapy.

Currently, most Dutch centers use a T-cell replete allogeneic SCT regimen with post-transplant GvHD prophylaxis consisting of cyclosporine A (CyA) and mycophenolate mofetil (MMF). With this regimen the incidence of acute GvHD II-IV is 50-60% [24-28]. Recently, several groups have reported efficacy data of post-transplant GvHD prophylaxis consisting of high-dose cyclophosphamide given on days +3 and +4[29, 30][31-33]. The rationale for this strategy is that alloreactive donor T lymphocytes are activated after the infusion into the recipient, enter a proliferative phase, and are thus sensitive to the cytotoxic effect of cyclophosphamide 72 hours later. On the other hand, non-alloreactive, non-proliferating T-cells are spared and may provide protection against infections in the short term and allow for a more robust immune reconstitution. Engraftment is unaffected, since hematopoietic stem cells express high levels of aldehyde dehydrogenase, rendering them resistant against the cytotoxic effects of high-dose cyclophosphamide[34]. In the setting of T-cell replete haplo-identical SCT, post-transplant high-dose cyclophosphamide was combined with conventional immunosuppression consisting of a calcineurin inhibitor plus MMF and resulted in an incidence of acute GvHD of 12-34% [29, 31-33]. In addition to these low rates of acute GvHD, the incidence of NRM was shown to be very favourable as well (7-18%)[29, 31-33]. In the setting of fully matched related or unrelated donor transplantation, post-transplant high-dose cyclophosphamide has been used as the sole form of GvHD prevention and resulted in an incidence of acute GvHD of 43% with a NRM rate of 15% at 2 years[30]. Such a strategy of a short-course prophylaxis of GvHD is meant to enable the implementation of early post-transplant chemotherapy, without facing possible drug interactions.

In recent years, the Leiden team has used early donor lymphocyte infusions to induce GVL at the post-transplant timepoints of either 3 or 6 months. Low dose DLI at the 3 month time point (0.3×10^6 CD3+ T cells/kg in related, 0.15×10^6 CD3+ T cells/kg in T-cel depleted unrelated donor SCT) was studied in a group of 20 patients. Out of the 18 patients evaluable, 22% developed overall grade 1-2 acute GvHD and 6% overall grade 3-4 acute GvHD. Acute GvHD resolved in all patients, two patients needed systemic prednisolone. Most patients received further DLI dose escalation at 6 months after alloHSCT. With a median follow up of 3.2 years (range 0.5-3.8), overall survival in this group of high risk patients appeared encouraging and estimated approximately 70%. It was concluded that the administration of low dose DLI early after SCT was safe and accompanied by encouraging GVL-effects.

5.3 Overview and safety profile of decitabine

Several clinical trials investigating different drug dosing schedules of decitabine demonstrated significant clinical benefits in the treatment of patients with MDS and AML [35-38]. Decitabine was initially investigated in a trial of 10 MDS patients [39, 40]. Patients were treated with decitabine at a daily dose of 45 mg/m2 divided into three four-hour infusions for three days (six patients) or as a continuous infusion of 50 mg/m2 for three days (four patients). Decitabine induced an overall response rate of 50% with a complete hematologic response in 40% of patients. A subsequent clinical trial reported similar response rates [40]. The low-dose decitabine was studied in a phase I study with various low-dose levels in patients with MDS or leukemia [38]. Pharmacodynamic studies revealed that dose-dependent decreases in DNA methylation reached a plateau at approximately 150 to 200 mg/m^2/course [41]. These results suggested that response to decitabine can occur at levels below the MTD and that prolonged exposure increases the response.

Decitabine was approved by the Food and Drug Administration (FDA) in 2006 for the treatment of MDS on the basis of a phase III multicenter trial [42]. In this trial, 170 patients with MDS were randomized to receive either i.v. decitabine 15mg/m^2 over three hours every eight hours for three consecutive days (135 mg/m^2/course) every six weeks or the best support- ive care (BSC). The overall response rate for the decitabine arm was 30%, compared with 7% for the BSC arm (P <0.001). The median duration of the response was 41 weeks with no difference in the time to AML progression compared with the BSC [42]. Similarly, the European Organization for Research and Treatment of Cancer and the German MDS Study Group conducted a large phase III multicenter trial that randomized 233 elderly patients with higher-risk MDS to receive either i.v decitabine 15 mg/m2 over three hours every eight hours for three consecutive days (135mg/m^2/course) every six weeks or the BSC[43]. This trial reported a 34% overall response rate with decitabine and an improvement in the progression- free survival (0.55 versus 0.25 years; P = 0.004) compared with the findings for the BSC group.

The time to AML progression and overall survival, however, did not differ significantly between the two groups. The main adverse effect due to decitabine treatment appears to be myelosuppression, including severe neutropenia, thrombocytopenia, and anemia. The incidence of myelosuppression, however, decreases in responding patients with the continuation of therapy. Grade 3/4 non-hematologic toxicity is rare and usually clinically insignificant.

In a study of elderly AML patients, decitabine was administered at 20 mg/m2/day for five days every four weeks [44]. The overall response rate was around 26%. The median time to response was three cycles. Toxicities were similar to those in the previous studies at this dose level. A phase III study that enrolled 485 patients who were at least 65 years of age with newly diagnosed, de novo, or secondary AML and intermediate or unfavorable risk cytogenetics was performed [45]. Decitabine was given at a dose of 20 mg/m^2/day as a one-hour infusion for five consecutive days every four weeks. Patients on decitabine had a median overall survival (OS) of 7.7 months, compared with 5 months in the control

arm with a hazard ratio of 0.85. The stratified log-rank analysis, however, did not demonstrate a statistical significance between the groups. Subsequently, an unplanned OS analysis with one year of additional follow-up demonstrated the same improvement in median OS with a nominal P value of 0.037.

5.4 Decitabine before or after allogeneic HSCT

The role of therapy with hypomethylating agents such as decitabine upfront to bridge the time to allo-HSCT has been investigated by several sites during recent years (Table 2).
De Padua Silva and coworkers [46] reported on (17) patients with MDS (n =11) and AML (47) (n =6) who received decitabine as part of the phase II clinical trial conducted by Kantarjian et al [36] and proceeded to allo-HSCT. A median number of five courses of decitabine was given. Most patients had higher-risk MDS and poor-risk cytogenetics; median age was 55.5 years (range, 36 – 66 years). Conditioning regimens were based on combination of fludarabine with busulfan or with melphalan. At time of transplant, seven patients were in CR, whereas 10 had evidence of disease. A total of 16 patients engrafted and 13 (76%) had complete donor chimerism at day 100 after allo-HSCT; six of them were transplanted in CR.
Patients with AML or MDS scheduled for allo-HSCT are mostly treated with induction chemotherapy up-front to transplantation in order to control the disease and gain time for identifying a matching donor. However, induction chemotherapy is often followed by adverse effects like prolonged cytopenia and severe infections, especially in older patients. Therefore Lübbert et al [47] designed a milder treatment approach (“inDACtion” instead of induction therapy) prior to allo- HSCT with a reduced-toxicity conditioning regimen.
The authors reported on 15 older patients (median age, 69 years; range, 60–75 years) with MDS (n= 10) and AML (n=5) who were treated with decitabine within a phase II multicenter study (PCH 95–11) of decitabine in advanced MDS (n=3),[48] the MDS phase III study 06011 of the EORTC/German MDS Study Group (n=7; described above),[43] and the multicenter phase II study 00331 of older patients with AML (n=5).[49]The median number of decitabine courses given was five, with attainment of CR in five patients and PR in one patient as best response. Patients were treated prior to allografting with a reduced-toxicity conditioning according to the FBM-protocol (fludarabine, BCNU, melphalan).[50-52]Stem cell donors were matched siblings in four patients and unrelated donors in 11 patients (four with HLA mismatch). Allografting was performed as a consolidation at the time of best response to decitabine, as a salvage therapy for relapsed or refractory disease. Overall, 14 patients engrafted (one patient died due to infection on day=13, before engraftment) and eight (53%) had a complete donor chimerism 100 days after allo-HSCT. In summary, this analysis revealed that treatment with decitabine followed by reduced-intensity conditioning by FBM protocol and allogeneic transplantation is feasible and safe. Recently, Kim and coworkers [53] published their observations on 19 younger patients (median age, 47 years; range 23– 69 years) with MDS (n=16) and AML (n =3)

who received either azacitidine (n=10) or decitabine (n =9) before alloHSCT. The median number of treatment cycles with hypomethylating agents was three. Eight patients (43%) achieved CR and mCR; notably CRs were more frequent in patients treated with decitabine compared to azacitidine-treated patients (66% *v* 20%, respectively). Allo-HSCT conditioning consisted mostly of a non-myeloablative regimen (n=18). Stem cell donors were matched siblings in seven patients (37%), haploidentical family members in five patients (26%), and unrelated donors in seven patients (37%). A total of 18 patients engrafted after a median time of 13 days; this was significantly shorter in decitabine-treated patients (engraftment after decitabine 11.9 days *v* 16.6 days after azacitidine treatment, *P* <.005). Patients in the decitabine group showed a higher incidence of acute graft-versus-host disease (67% *v* 20% in the azacitidine group, *P* <.055). The overall survival rates after 1 and 2 years were 95% and 68%, respectively, with no significant differences between the azacitidine and decitabine groups after 1 year.

The investigators of all four trials concluded that upfront treatment with decitabine prior to allo-HSCT in patients with MDS is a feasible and effective strategy for bridging at the time of donor search and simultaneously controls and “downstages” the disease. Toxicity does not seem to be increased by previous use of hypomethylating agents.[46, 47, 53]

Furthermore, two phase I trials in the United States and Korea are recruiting patients to explore whether decitabine is a safe and tolerable maintenance therapy for patients with MDS or AML after alloHSCT, that may reduce relapse rate (NCT00986804; NCT01277484).

5.5 Overview of panobinostat (LBH589)

Panobinostat (LBH589) is a potent class I/II pan-DAC inhibitor (pan-DACi) that has shown anti-tumor activity in pre-clinical models and cancer patients. Deacetylases (DAC) target lysine groups on chromatin and transcription factors and various non-histone proteins such as p53, tubulin, heat shock protein 90 (HSP90) and retinoblastoma protein (Rb). Panobinostat is formulated as an oral capsule and a solution for intravenous (i.v.) injection. A summary of key clinical data on panobinostat is herewith reported, for details please refer to the Investigator Brochure.

In patients oral panobinostat is rapidly absorbed and maximal plasma concentrations are reached within one hour. Its bioavailability is approximately 40% and the elimination half-life is ~ 16 hours. Using intermittent, i.e. three-times-a-week weekly, administration schedule, the time to reach steady-state panobinostat plasma level is approximately seven days. Oral panobinostat as a single agent has been tested in hematological malignancies in a large phase I study [CLBH589B2102]. This study was planned to evaluate 2 schedules, including a (Arm1) three-times-a-week every week schedule and a (Arm 2) three-times-a-week every other week schedule. The MTD with the three-times-a-week weekly schedule of single agent panobinostat in patients with leukemias/MDS was declared to be 60 mg. Across Arm 1 and 2, a total of 4,834 post-dose ECGs were performed in 146 enrolled patients. No dose dependent increase in mean change from baseline QTcF was noted. Five patients (3%)

experienced QTcF >500 ms. However, all five patients were among the 76 patients (7%) treated at dose levels ≥60 mg. A preliminary evaluation of clinical data from 71 patients with AML indicated that no antileukemic activity was observed in patients treated in Arm 2, and that no antileukemic activity was noted in dose levels of ≤40 mg/day, even with the three times-a-week weekly regimen. Thirty-six AML patients were treated in Arm 1 (three-times-a-week every week) at dose levels ≥40 mg. CR was reported in 2 (6%) and PR in 1 (3%) AML patients with prior MDS treated at 60 mg. Prolonged SD was reported in additional 2 (6%) AML patients with prior MDS, lasting for more than 14 months. Another patient treated at the 60 mg dose level had no evidence of anti-leukemic effect during treatment, lasting 3 months. CR was reported 1 month after end of treatment. A patient treated at 40 mg had stable disease during study treatment which lasted for 2 months (Ottmann et al ASH-2008). Stabilization of disease for 20 cycles with decrease in bone marrow blasts was observed in one MDS patient out of 7 treated with the three-times-a-week every week schedule.

5.6 Combination of DNA methyltransferase inhibitors with deacetylase inhibitors

Widespread epigenetic modifications affecting gene expression are well documented in MDS and AML, and hypermethylation of promoter regions of numerous genes have been shown to play an important role in tumorogenesis. The physiologic relevance is that silencing of some genes enables malignant cells to gain a selective advantage, leading eventually to immortality, invasion, and the development of a neoplastic phenotype. DNA methylation and histone acetylation are independent and modulate gene expression. In fact, chromatin status is determined by chemical modifications occurring directly on DNA. Methylation recruits methyl-binding proteins locally and the latter bind protein complexes containing corepressors, DNA methyltransferases (DNMTs) and histone deacetylases. In cancer, repression of gene expression is maintained by corepressor complexes of proteins recruited by regionally hypermethylated DNA. Whereas aberrant DNA methylation patterns, changes in chromatin structure and in gene expression are common in different tumor types, studies in leukemias have provided paradigmatic examples for the functional implications of genetic and epigentic alterations in cancer development. In hematological malignancies, methylation turns off tumor suppressor genes to achieve an effect similar to (or complementary to) gene mutations. Perturbation of the balance of transcriptional regulators may lead to aberrant recruitment of histone deacetylase (HDAC) and silencing of tumor suppressor genes (reviewed by Baylin and Jones [54, 55]. Complexes involving DNA methyltransferase and HDACs work in coordination to remove acetyl groups from histone tails and add methyl groups to CpG dinucleotides within gene promoter regions, thereby suppressing gene transcription. Inhibiting each of these enzyme complexes may synergistically remove this aberrant silencing, thus contributing to re-expression of essential tumor-suppressor genes. Therefore the concept of combining demethylating agents with DAC inhibitors - both epigenetic modifiers - provides a sound rational strategy. On this biological background, combination therapy with hypomethylating agents and DAC inhibitors has been attempted.

Combination strategies involving either drug sequencing or co-administration of these agents in vitro have shown improved activity with greater expression of previously silenced genes. In addition clinical studies in hematologic malignancies published to date demonstrate feasibility of the association of the two types of agents in these disease settings.

Patients with AML have also received decitabine in combination with other drugs. Decitabine has been administered to subjects at doses of 15 to 500 mg/m2 in combination with other drugs including: busulfan [56], amsacrine [57], idarubicin [57], daunorubicin [58], and cyclophosphamide [56] . Several studies have also explored the combination of decitabine with histone deacetylase inhibitors, another form of epigenetic therapy ([59-61]. Table 2 summarizes dosing and efficacy details from those studies.

Table 1: Dosing Regimens and Response Rates for Subjects With AML; Combination Therapy

Study No.	No. of Patients (N)	Starting Dose; Regimen	Response
Willemze 1997[57]	63 (AML + ALL)	DAC 125 mg/m^2 IV over 6 hrs q 12 hrs x 6 days plus m-Amsa 120 mg/m^2 IV over 1 hr days 6 and (n=30) or DAC 125 mg/m^2 IV over 6 hrs q 12 hrs x 6 days plus idarubicin 12 mg/m^2 IV over 15 mins Days 5, 6, and 7 (n=33)	Decitabine / m-Amsa: CR 8 (27%) Decitabine / Idarubicin: CR 15 (45%)
Garcia-Manero 2006[60]	AML: 48 MDS: 6	DAC: 15 mg/m^2 IV daily x10 days VPA (3 dose levels) 20, 35, and 50 mg/kg po x 10 days	DAC + 20 mg/kg VPA: 1 w/CR (33%) after 2 COT DAC + 35 mg/kg VPA: 1 w/CR (11%) after 3 COT DAC + 50 mg/kg VPA: 8 w/CR 2w/CRp (23%) after 1 COT
Willemze 1991[62]	17 ALL + AML (refractory = 5; relapsed = 12)	200–500 mg/m^2/day x 4-6 days alone OR combined with m-Amsa	DAC/m-Amsa:8/12 relapsed (67%) with CR No major antileukemic effects in refractory pts, Significant toxicity
De Lima 2003[56]	AML: 12 pts MDS: 1 pt ALL: 1 pt CMML: 1 pt CML: 9 pts	DAC: 400, 600, 800, or 1000 mg/m^2 (IV) Busulfan: 1 mg/m^2 q6h x 12 doses (po) Cyclophosphamide: 100-120 mg/m^2 Followed by Allogeneic SCT	9/24 pts alive at median of 17.1 mos; 7/24 pts disease free at median of 8.7 mos; 40% AML patients alive & disease-free at 3 years. Treatment-related mortality 35% at 3 years
Schwartsmann 1997[58]	8 pts	DAC90mg/m^2 IV over 4hrs x 5 days + daunorubicin 50 mg/m^2 IV x 3days;q4-6wks	5 CR after single COT; 1 CR after 2 COT; remission duration of 5 – 24 mos
Blum 2007[59]	25 (12 untreated; 13 relapsed)	DAC alone from 15 to 20 mg/m^2 /d IV over 1 hr x 10 days (n=14) Or DAC 20 mg/m^2 daily x10 days, plus VPA from 15 to 25mg/kg/day po x Days 5-21(n=11)	Optimal biologic dose of DAC= 20 mg/m^2/day; 44% response rate (11/25): 4 CR, 4 CRi, 3 PR.
Ravandi 2007[36]	31 (21 AML)	DAC 10, 15, 20 and 25 mg/m^2 /d IV x 5 days SAHA 100mg po tid x 14 days (1st cohort) & 200mg po tid x 14 days (all other cohorts)	1 CR/30 evaluable pts & 4 with significant reductions in blasts

Study No.	No. of Patients (N)	Starting Dose; Regimen	Response
Yee2007[63]	27	DAC20mg/m^2 IV dailyx 5days Vorinostat (SAHA) 100 mg bid or 200 mg bid Days 1-21 or 200 mg tid Days 1-14	1 CRi+1 MLFS+3 PR/ 25 evaluable pts
Issa 2008[64]	76 (23 AML)	DAC 20 mg/m^2 IV daily x 5 days q4 weeks vs. DAC at a similar dose plus VPA 50 mg/kg po for 7 days	Overall RR 46% with CR in 23/67 (34%). Overall RR in AML pts = 39%
Kirschbaum 2009[65]	61 evaluable (11 MDS) (25 relapsed AML) (25 untreated AML)	DAC 20 mg/m^2 /d IV over 1 hr x 5 days Vorinostat 400mg QD for 7-14 days	18% Response (CR + CRi) in MDS, 8% (CR + CRi) in relapsed AML, 36% (CR + CRi) in untreated AML

Notes: ALL = acute lymphoblastic leukemia; AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; CMML = chronic myelomonocytic leukemia; COT = course of treatment; CR = complete remission; CRi = CR without complete recovery of counts; CRp = CR except platelet count increase by 50% to >30 X 109 but <100 X 109/L; DAC = DACOGEN; HDAC = histone deacetylase; HI = hematological improvement; m-Amsa= amsacrine; MDS = myelodysplastic Syndrome; MI = myocardial infarction; MLFS = morphological leukemia-free state; PR = partial remission; pts = patients; RAEBT = refractory anemia with excess blasts in transformation; RR = remission rate; SAHA (Vorinostat) = Suberoylanilide Hydroxamic Acid; tid = three times a day; VPA = valproic acid, CTX =cyclophosphamide

Overall, the clinical experience on the use of epigenetic therapy in hematological disorders, suggests that this therapeutic approach is safe, well tolerated, and induces higher response rates compared to the single agent

5.7 Rationale of the study

This study will explore the feasibility of post-transplant panobinostat combined with decitabine after reduced intensity conditioning alloHSCT in AML patients. While recent studies showed that the allogeneic graft-versus-leukemia effect is clearly operational in poor-risk AML, relapse rates after alloHSCT in those patients are still unacceptably high, with no curative options left. Two new recent developments provide new avenues to explore further. First, timing of alloHSCT more early, ie as soon as possible after mucosal recovery from the preceding cycle of chemotherapy, and secondly the continued application of low dosages of chemotherapy after alloHSCT aimed at restoring epigenetic dysregulation and thereby creating a window to optimally exploit the allogeneic GVL-effect by escalating donor lymphocyte infusions. The strategy of a short-course intensified prophylaxis of GvHD by use of cyclophosphamide, of which the feasibility and efficacy have been demonstrated elaborately before is applied to enable the implementation of early post-transplant chemotherapy, without facing possible drug interactions. Based on recent experience by others exploring the combination of panobinostat and decitabine in AML patients and by different groups exploring post-transplant chemotherapy including panobinostat, we here propose to study the aforementioned combination of epigenetic therapy after alloHSCT to be followed by donor lymphocyte infusions in a carefull stepwise

approach to optimally profit from the allogeneic GVL-effect. Feasibility in this study will be defined by the completion of protocol treatment up to eligibility for a first dose of DLI in more than 70% of patients starting protocol treatment, without dose limiting toxicity up to that point of time.

6 Study objectives

Primary objective

Part I

- To asses the safety and feasibility of post-transplant panobinostat combined with decitabine to a regimen of T-cell replete RIC alloHSCT in patient with very poor-risk AML/RAEB, and select the recommended dose level for part II of the study

Part II

- To assess the feasibility and efficacy of addition of post-transplant panobinostat combined with decitabine to a regimen of T-cell replete RIC alloHSCT and DLI in patients with (very) poor-risk AML/RAEB

Secondary objectives

To assess efficacy in terms of:

- Complete remission rate at 3, 6, 12, and 24 months post alloHSCT
- Relapse/progression rate at 3, 6, 12, and 24 months post alloHSCT
- Overall survival (OS) from study registration as well as OS from start protocol treatment
- Progression free survival (PFS) from alloHSCT with relapse (for patients in CR) and progression (for patients in PR) as events
- Engraftment and chimerism at 3, 6, 12, and 24 months post alloHSCT

To assess toxicity in terms of:

- The incidence and nature of (serious) adverse events
- The incidence and severity of acute and chronic GvHD up to 24 m post alloHSCT
- NRM up to 24 months post-transplant
- Number and percentage of registered patients starting protocol treatment (eligible for alloHSCT)
- Number and percentage of patients receiving post-transplant epigenetic therapy after alloHSCT
- Number and percentage of patients receiving DLI after alloHSCT

7 Study design

The trial is designed as a prospective, multicenter phase I/II feasibility trial. The study is intended to be performed in both poor-risk and very poor-risk patients following evaluation and discussion of the interim analysis by both the DSMB and writing committee. However, part I of the study will exclusively be performed in very poor-risk patients and patients refractory to the first cycle of AML induction therapy.

Poor-risk and very poor-risk AML/RAEB patients will be registered during the first course of chemotherapy, or shortly thereafter if refractory. Registration also implies that a donor search (related or unrelated) should be feasible. Immediately following registration, the first step is to start a donor search with the aim to perform a transplant within 6-8 weeks. This may imply that a search among family members and an unrelated donor search in Bone Marrow Donors Worldwide (BMDW) is started simultaneously. Upon registration of the patient within this study, the urgency of the unrelated donor search will be forwarded to Europdonor, that will then perform the first inventory immediately.

All registered patients, whether or not in remission after the first cycle of chemotherapy, will proceed to a second cycle of chemotherapy that includes high dose cytarabin, preferably according to the second cycle of the ongoing HOVON-SAKK protocol. Upon recovery of mucositis and resolution of opportunistic infections, patients in principle qualify for proceeding to alloHSCT, provided that a bone marrow sample has shown at least responsive disease as evidenced by less than 10% blasts. At that moment in- and exclusion criteria for alloHSCT and subsequent chemotherapy will be checked. Following compliance with in-/ex-clusion criteria and providing informed consent, protocol treatment will start.

As the toxicity and feasibility of post-transplant chemotherapy will depend on the preceding conditioning regimen and GvHD prophylaxis, this study will avoid the use of many different conditioning regimen and focus on only one RIC regimen and one intensified schedule of GvHD prophylaxis that incorporates also cyclophosphamide after transplantation.

AlloHSCT will be performed after conditioning with intravenous cyclophosphamide (14.5 mg/kg/day) on day -6 and -5, intravenous fludarabine (30 mg/m^2/day) on days -6 to -2 (5 days), and low dose (2 Gy) TBI on day -1. At day +3 and +4 intravenous high-dose cyclophosphamide 50 mg/kg/day will be given. Thereafter, from day +5 until day +70 cyclosporine A will be given intravenously based on trough levels. Trough levels are targeted to the upper therapeutic range [250-350 µg/L using the immunoassay and 200-300 µg/L using HPLC] and monitored at day 0, day +3, day +7 and thereafter once a week. Cyclosporine A will be stopped at day +70 without tapering.

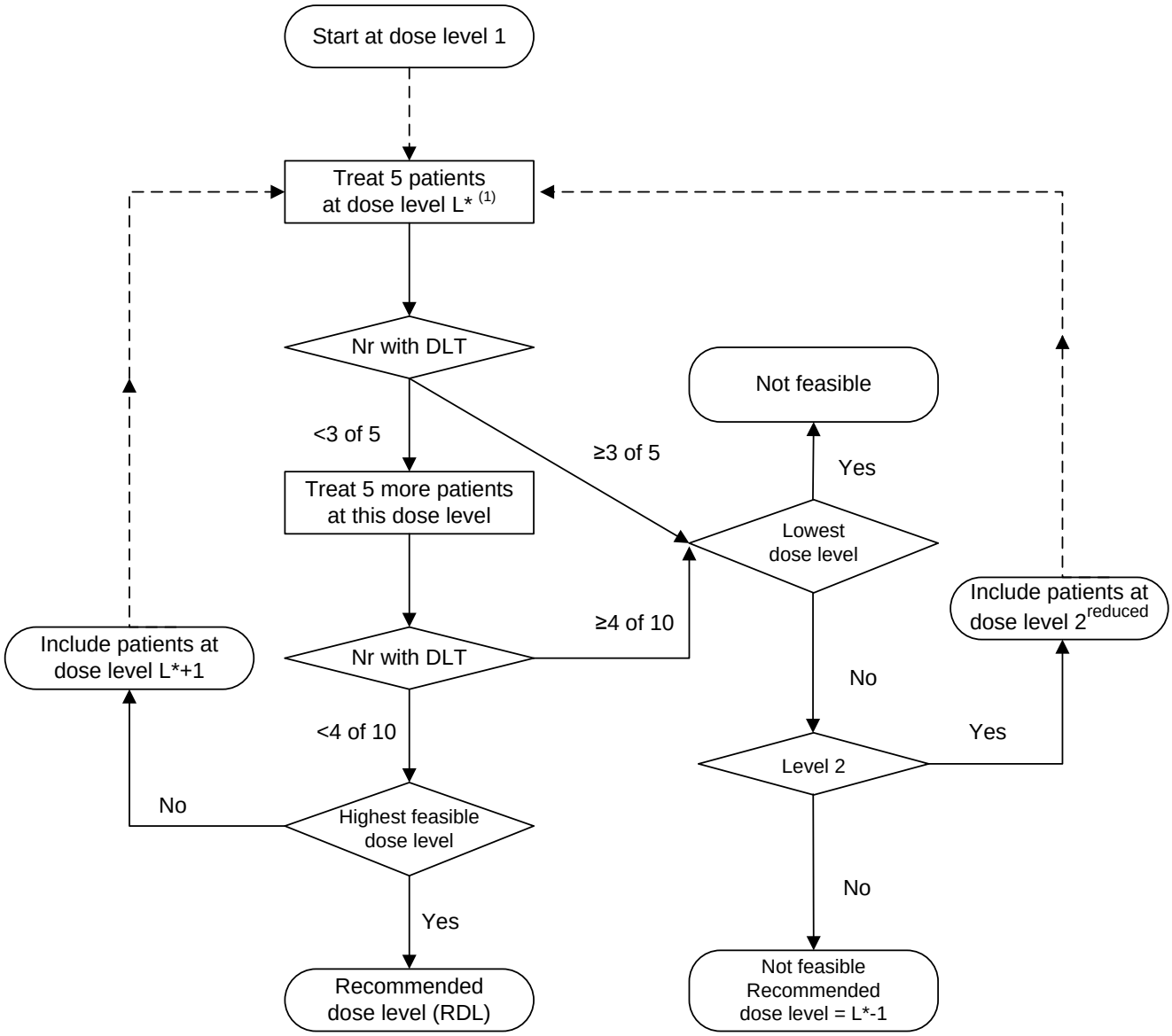
After transplantation, patients will receive two 28-days courses of panobinostat and decitabine starting at day 28 after alloHSCT. Shortly after the last course, all patients will receive a low dose DLI at day 90 - 100 after alloHSCT. Subsequently another 2 courses of the combination of panobinostat and azacitidine will be given as from day 21 after DLI. At day 180 - 200 after alloHSCT all patients will receive a second dose (and third) of donor lymphocytes, if no prior GvHD exceeding grade I, limited to skin-GvHD, has occurred.

A total of 100 registered patients will be included in this study.

Part 1) Dose level selection panobinostat/decitabine
The combination of panobinostat and decitabine, post alloHSCT, will be tested at 4 dose levels (see page 9.3.2), with the goal to establish the recommended dose level for part 2 of the study.

The first dose level will be Panobinostat alone, to be tested in 10 patients. These 10 patients will be evaluated for DLT associated with Panobinostat alone and will also be evaluated to see whether a sufficient number of patients may proceed to epigenetic therapy due to absenece of GVHD, thereby allowing to evaluate safety and efficacy of post-transplant PNB/DAC in a sufficient number of patients. In order to efficiently asses feasibility of post-transplant PNB/DAC, 7 out of 10 patients should be eligible to continue with epigenetic treatment. Therefore, the incidence of acute GvHD at 1 month should not exceed 30%. The DSMB will be presented with the results with respect to the incidence of acute GvHD and DLT of the first 10 patients and will provide their opinion as regards toxicity, incidence of GvHD, and penetration to epigenetic therapy (see stastitical paragraph).

Decisions regarding feasibility and dose escalation to the next cohort, continuation or stopping are based on the dose limiting toxicity (DLT, described in section 7.1). Enrollment at each dose level will consist of a maximum of 10 patients. Patients who die of leukemia within 35 days after start of PNB/DAC cycle 1, but without a DLT, will be considered to be non-evaluable, and will be replaced by other patients.
The dose escalation stops as soon as at least three patients experience a DLT in the first cohort of five patients treated at that dose level, or at least four in the two cohorts of five patients treated at that dose level or, alternatively, when the highest planned dose level has been reached. Before opening the next higher dose level the DLT information at the preceding dose level will be reviewed and escalation will be undertaken as appropriate.
.
Part 2) The recommended dose level (RDL) for part II of the study is defined as the highest dose level with 3 or less DLTs observed during cycle 1 among 5 patients.



(1) L* should be read as `1' or `2', whichever applicable

The DSMB will advise the principal investigator and the study coordinators and writing committee on the determination of the recommended dose level.

Details of all treatments (dose and schedule) are given in paragraph 9.

7.1 Definition of dose limiting toxicity

Dose limiting toxicity is defined as

- Grade 4 non-hematological toxicity between start cycle 1 PNB/DAC and start cycle 2 PNB/DAC or day 35 (whichever occurs first)

- Hematological toxicity which results in delay of the start of cycle 2 after day 35 (as described in section 9.3.2)
- TRM between start cycle 1 PNB/DAC and start cycle 2 PNB/DAC or day 35 (whichever occurs first)

A patient who did not receive cycle 1 PNB/DAC is considered not evaluable for DLT and will be replaced.

8 Study population

8.1 Eligibility for registration

All patients must be registered before start of the second cycle of chemotherapy (either re-induction or first consolidation) and must meet all of the following eligibility criteria.

8.1.1 Inclusion criteria

- Patients with poor-risk or very poor-risk AML or RAEB with IPSS ≥ 1.5 , (see appendix D). During the phase I part only very poor-risk patients will be included
- Eligibility for continuation with intensive induction/consolidation chemotherapy
- Eligible for allogeneic donor search (related/unrelated)
- 18-70 years, inclusive
- Negative serum pregnancy test for female patients of childbearing potential, at registration
- Female patients of childbearing potential must use an effective contraceptive method during the study and for a minimum of 6 months after study treatment
- Written informed consent

8.1.2 Exclusion criteria

- History of active malignancy during the past 2 years with the exception of basal carcinoma of the skin or carcinoma “in situ” of the cervix or breast
- Known HIV-positivity
- Pregnant or breast-feeding female patients

8.2 Eligibility criteria for start of protocol treatment

8.2.1 Inclusion criteria

- Poor-risk or very poor-risk AML or RAEB with IPSS ≥ 1.5.
  During the phase I part only very poor-risk patients will be included.
- Responsive disease (< 10% blasts at 3 and/or 4 weeks after start of induction cycle II)

- Recovery of mucositis after preceding chemotherapy
- Absence of active opportunistic infections
- Absence of active CNS localisation
- HLA-compatible donor available (≥ 7/8 matched unrelated donor or fully matched sibling donor)
- WHO-performance status 0-2
- Written informed consent

8.2.2 Exclusion criteria

- Severe cardiac dysfunction (NYHA classification III-IV, see appendix H)
- Severe pulmonary dysfunction (CTCAE grade III-IV, see appendix G)
- Severe neurological or psychiatric disease
- Significant hepatic dysfunction (serum bilirubin or transaminases ≥ 5 times upper limit of normal)
- Significant renal dysfunction (creatinine clearance < 30 ml/min after rehydration)
- Any psychological, familial, sociological and geographical condition potentially hampering compliance with the study protocol and follow-up schedule
- Concurrent severe and/or uncontrolled medical condition (e.g. uncontrolled diabetes, infection, hypertension, cancer, etc.)

9 Treatment

9.1 Donor search

Following registration, an HLA-identical donor search must be initiated as soon as possible, first among siblings and secondly in the world donor bank, inventory in BMDW is to be performed simultaneously with family typing. In order to avoid inappropriate delay in case no suitable sibling is present, high-resolution HLA typing should be performed immediately after registration enabling a more rapid matched unrelated donor search. A suitable matched unrelated donor must have at least a ≥ 7/8 allele match (HLA A, B, C, DRB1). Only HLA C mismatches are allowed in case of a 7/8 matched donor.

The urgency of the unrelated donor search will be forwarded to Europdonor upon registration of the patient within this study, that will then perform the first inventory immediately.

9.2 Reduced intensity allogeneic SCT

Patients must meet the eligibility criteria as described in section 8.2. Preferably, RIC alloHSCT is performed within 4-6 weeks (but no later than 9 weeks) after start of the last chemotherapy in patients without active infections and mucositis at that point of time. It implies that RIC allo HSCT will be performed in patients without hematological recovery.
The allogeneic transplantation is performed after conditioning with intravenous cyclophosphamide, fludarabine and low dose TBI. At day +3 and +4 intravenous high-dose cyclophosphamide 50 mg/kg/day will be given. Thereafter, from day +5 until day +70 cyclosporine A will be given intravenously based on trough levels. Trough levels are targeted to the upper therapeutic range [250-350 µg/L using the immunoassay and 200-300 µg/L using HPLC] and monitored at day 0, day +3, day +7 and thereafter once a week. Cyclosporine A will be stopped at day +70 without tapering.

Agent	**Dose/day**	**Route of administration**	**Days**
Cyclophosphamide	14.5 mg/kg/day	Intravenously	-6 and -5
Fludarabine	30 mg/m2/day	Intravenously	-6 to -2 (5 days)
Low dose TBI	2 Gy		-1
Stem cell infusion			day 0
Cyclophosphamide	50 mg/kg/day	Intravenously	+3 and +4
Uromitexan	4 dd 20 mg/kg	Intravenously	+3 and +4
Cyclosporine A	3 mg/kg/day	Intravenously	+5 to +70

9.2.1 Special management in conjunction with allogeneic transplantation

- Stem cell collection in related donors: donors can be mobilized according to local protocols.
- As an alternative to the preferred source of stem cells (peripheral blood stem cells), also bone marrow is allowed, according to local protocol.
- HLA matching between patient and donor: HLA identical sibling donors 7/8 or 8/8 matched unrelated donors (for HLA-A, B, C, DR) are allowed.
- In case of major ABO blood group incompatibility and a high load of red blood cells (>200x10^9), and/or an anti-A or anti-B titer of ≥ 1/16: prehydration with 1L NaCl 0.9% over 4 hrs and slow starting of stem cell graft infusion, starting with 1 ml/min, to be doubled after 30 min. and then further increased to 3 ml/min if no adverse reactions occur (or according to local protocol).

9.2.2 Cryopreservation of cells from the donor graft for DLI (I+II)

A small fraction of the graft should preferably be used to freeze T cells for DLI at days 100 and 180. The first DLI will consist of 1 x 10^6 T cells/kg, in case of a sibling donor, and 0.3 x 10^6 T cells/kg in

case of an unrelated donor. The second DLI will consist of 3 x 10^6 T cells/kg in case of a sibling and 1.5 x 10^6 T cells/kg in case of an unrelated donor.

- Measure absolute numbers of $CD34^+$ HPC and $CD3^+$ T cells in the graft by flowcytometry.
- Under the condition that the graft still contains at least 2 x 10^6 $CD34^+$ cells/kg after removal of the fraction for DLI, harvest a volume containing 3 x 10^6 $CD3^+$ T cells/kg in case of an unrelated donor or 10 x 10^6 $CD3^+$ T cells /kg in case of a related donor, taking into account the expected average loss of viable $CD3^+$ T cells by the local freezing and thawing process. In general, a loss of 50% may have to be taken into account.
- Cryopreserve the T cells fraction in (at least) two parts with an expected recovery after thawing of 0.3 x 10^6 viable T cells/kg and 1.5 x 10^6 viable T cells/kg in case of a unrelated donor or with an expected recovery after thawing of 1 x 10^6 viable T cells/kg and 3.0 x 10^6 viable T cells/kg in case of a related donor. Cryopreserve two reference vials to determine the recovery of viable $CD3^+$ T cells after thawing.

Note: the third DLI will be harvested and administered fresh.

9.2.3 Infection prophylaxis after transplantation

Prophylaxis against bacterial and fungal infections and Pneumocystis jirovecii pneumonia will be performed according to local practice. As a minimal prophylaxis co-trimoxazole and valacyclovir is advised during the first year after transplantation. Monitoring of CMV and EBV will be performed following standard procedures and pre-emptive therapeutic intervention will be initiated when appropriate. It is advised to perform weekly CMV and EBV monitoring during the first 90 days after transplantation.

9.2.4 Therapy of CMV reactivation

Valganciclovir 900 mg PO twice daily for 14 days. In case of insufficient response further treatment with intravenous ganciclovir or foscarnet (or according to local protocol).

9.2.5 Therapy of EBV reactivation or post transplant lymfoproliferative disease (PTLD)

Rituximab 375 mg/m^2 will be administered for EBV copy numbers in peripheral blood of >1000 geqs/ml (3 log EBV count). Repeat if EBV copies are higher than 50% of starting level after 72 hrs. (or according to local protocol)

9.2.6 Staging and handling of Graft versus Host Disease

Acute and chronic GvHD will be staged according to the criteria described in appendix I

Acute GvHD grade ≥ 2 should be treated with prednisone (2 mg/kg PO or IV for 10 days). In case of complete GvHD response, reduce the dose with 50%. Thereafter prednisone should be tapered with 20 mg weekly until 40 mg/day. From that moment on, prednisone should be tapered with 10 mg weekly until a dose of 20 mg/day. Then the dose should be lowered with 5 mg weekly. CyA will be continued or restarted orally (9 mg/kg/day in 2 doses) or intravenously (3 mg/kg/day) if there is concern of GI absorption. Dosage of cyclosporin A should be based on trough levels. Steroid refractory acute GvHD is treated according to local guidelines. Extensive chronic GvHD (according to the NIH criteria in appendix I) is treated by combination immunosuppressive therapy, preferably prednisolone and cyclosporin or according to local guidelines. Chronic extensive GvHD or acute GvHD grades 3-4 excludes patients from receiving (further) combination therapy by panobinostat and decitabine.

9.3 Panobinostat and Decitabine post-transplantation

9.3.1 Eligibility criteria for post-transplantation panobinostat and decitabine chemotherapy

Inclusion criteria

- Absolute neutrophil count ≥ 1.0 x 10^9/L
- Platelet count ≥ 25 x 10^9/L
- Serum creatinine cleareance ≥ 30 ml/min
- Total bilirubin ≤30 µmol/l
- AST (SGOT) and ALT (SGPT) ≤ 3 x Upper Limit of Normal (ULN);

Exclusion criteria

- Severe cardiac dysfunction (NYHA classification II-IV, see appendix H)
- Severe pulmonary dysfunction (CTCAE grade III-IV, see appendix G)
- Severe neurological or psychiatric disease
- Significant renal dysfunction (creatinine clearance < 30 ml/min after rehydration)
- GvHD requiring the use of combination immunosupressive therapy
- Acute GvHD grades 3-4, chronic extensive GvHD
- Serious active infections
- CMV reactivation, which is not responsive to first line valganciclovir

N.B. CR after alloHSCT is NOT required to continue to panobinostat/decitabine treatment

9.3.2 Treatment schedule

Patients will receive 4 cycles of combination therapy of panobinostat and decitabine: cycle 1 and 2 after alloHSCT, cycle 3 and 4 after first DLI

Combination therapy is given according to the schedule below.

During the phase I part the patient will be treated at the applicable dose level at that moment.

Dose level 1

Agent	**Dose/day**	**Route of administration**	**Days**
Panobinostat	20 mg	Oral	1,4,8,11

Dose level 2 (intermediate dose)

Agent	**Dose/day**	**Route of administration**	**Days**
Panobinostat	20 mg	Oral	1,4,8,11
Decitabine	1 dd 20 mg/m^2	Intraveneus	1 to 3

Dose level 2 ^{reduced} (reduced dose)

Agent	**Dose/day**	**Route of administration**	**Days**
Panobinostat	20 mg	Oral	1,4,8,11
Decitabine	1 dd 10 mg/m^2	Intraveneus	1 to 3

Dose level 3 (escalated dose)

Agent	**Dose/day**	**Route of administration**	**Days**
Panobinostat	20 mg	Oral	1,4,8,11
Decitabine	1 dd 20 mg/m^2	Intraveneus	1 to 5

During the phase II part the patient will be treated according to the recommended dose level as determined during phase I.

The first cycle will start at day 28 after alloHSCT, or as soon as possible thereafter upon meeting the eligibility criteria as mentioned in section 9.3.1.

Cycle 2 is given at day 28 after start of cycle 1, or as soon as possible thereafter upon hematological recovery, requiring neutrophils ≥ 1.0 x10^9/L and platelets ≥ 25 x 10^9/L. *Do not give the next cycle at a reduced dose but delay if required.*

If cycle 2 cannot be started before day 100 after alloHSCT the patient will go off protocol treatment and will be treated with DLI according to local practice.

After the first DLI (see 9.4.2) patients will receive cycle 3 and 4 of combination therapy.
Cycle 3 will start at day 21 after DLI, or as soon as possible thereafter upon hematological recovery.
Cycle 4 will be given at day 49 after DLI, or as soon as possible thereafter upon hematological recovery.

9.3.3 Special management in conjunction with panobinostat and decitabine

- Additional prophylactic antibacterial antibiotics or tumor lysis prevention with allopurinol or hyperhydration do not have to be administered during panobinostat and decitabine therapy.

9.4 Post transplant donor lymphocyte infusion

9.4.1 Exclusion criteria for DLI:

- rapidly progressive disease
- any acute GvHD or chronic GvHD requiring the use of systemic immunosuppressive therapy
- unavailability of the donor for donation of peripheral blood for DLI in case no donor cryopreserved PBMC are available

9.4.2 Donor lymphocyte infusion

One to three DLI's are projected to be administered, depending on exclusion criteria for DLI.
DLI should only be administered a minimum of 14 days after the last administration of PNB/DAC.
DLI should be administered according to the schedule below.

	Dose (cells/kg)		**Days**
First DLI	Sibling donor:	1.0×10^6	day +90 – day +100 after alloHSCT
	Unrelated donor	0.3×10^6	
Second DLI	Sibling donor	3×10^6	day +180 – day +200 after alloHSCT
	Unrelated donor	1.5×10^6	
Third DLI	Sibling donor	1×10^7	> 2 months after 2nd DLI
	Unrelated donor	5×10^6	

The **first DLI** should preferably be given between day +90 and day +100 after alloHSCT.
The **second DLI** should preferably be given between day +180 and day + 200 after alloHSCT. If patients do not develop GvHD up to 2 months after the second DLI a **third DLI** should be administered.
For DLI using fresh PBMC, infusion will take place in the afternoon of the day of the PBMC collection.

Infusion of crypreserved DLI

Cryopreserved T cell products for DLI will be thawed and infused as per standard local practice for infusions of cryopreserved HPC, apheresis products. In case the actual recovery of viable $CD3^+$ T cells after thawing of the reference vial T cells strongly deviates from the average recovery of viable $CD3^+$ T cells, it is recommended to adjust the volume of the T cell product that will be infused accordingly (when possible).

In general, a loss of 50% of T-cells by the freezing and thawing procedure may have to be taken into account.

9.5 Patients with progressive disease after transplantation

Patients with progressive disease after alloHSCT should preferably continue with panobinostat and decitabine and DLI administration according to the protocol until at least 2 courses of combination therapy have been administered and the response to PNB/DAC and DLI can be evaluated.

9.6 Investigational Medicinal Products Panobinostat and Decitabine

9.6.1 Summary of known and potential risks

Panobinostat

Side effects include low blood counts, infections and bleeding. Also tiredness, anorexia, weight loss, dizziness, dyspnoea, headaches, artralgia, myalgia, pyrexia, alopecia, sleeplessness, hypokaliema, and gastrointestinal complaints (diarrhea, nausea, vomiting, constipation, dyspepsia) are seen. Many patients have reactions at the site of subcutaneous injection. Severe hypersensitivity reactions are seldomly seen (0.1-1%). On very rare occasions hepatic coma, kidney insufficiency and death have been reported. Please refer to the IB for more detailed information.

Decitabine

The most frequently reported adverse events in studies of decitabine in subjects with MDS, AML or chronic myelogenous leukemia (CML) include myelosuppression (neutropenia, thrombocytopenia) and consequent infections, and non hematologic adverse events (ie, nausea, vomiting, diarrhea, stomatitis, and alopecia). Generally these are manageable with the use of supportive measures and pharmacologic intervention. Please refer to the IB or SmPC for more detailed information.

9.6.2 Preparation and labeling

Panobinostat will be shipped to trial sites in containers labeled as an Investigational Medicinal Product. Panobinostat will be prepared and labeled by Novartis in compliance with GMP and other applicable regulatory requirements.

Decitabine will be shipped to trial sites in containers labeled as an Investigational Medicinal Product. Decitabine will be prepared and labeled in compliance with GMP and other applicable regulatory requirements.

9.6.3 Storage and handling

Panobinostat and decitabine should be stored and handled in accordance with the instructions in the Investigators Brochure: The investigational medicinal product should be stored in such a manner that accidental loss or destruction or access by an unauthorized person is prevented.

9.6.4 Study drug supply

The sponsor will arrange delivery of panobinostat and decitabine to trial sites. No investigational medicinal product will be shipped until the sponsor has verified that all regulatory required documents and approvals for the site are available.

9.6.5 Drug accountability

The investigator, or a pharmacist or other appropriate individual who is designated by the investigator, should maintain records of the product's delivery to the trial site, the inventory at the site, the use by each patient, 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.

9.6.6 Study drug return and destruction

Partially used investigational medicinal product should not be redispensed to either the same or another patient after it has been returned.
The trial site should destroy used or partially used study drug containers after drug accountability records have been completed. Destruction should be documented.
At the end of the trial or after expiry of the product unused investigational medicinal product should be destroyed by the trial site. Destruction should be documented.

10 Study procedures

10.1 Time of clinical evaluations

- Within 2 weeks before alloHSCT
- After alloHSCT
- After PNB/DAC cycle 1
- After PNB/DAC cycle 2
- After PNB/DAC cycle 3
- After PNB/DAC cycle 4
- 9, 12 and 18 months after alloHSCT
- 24 months after alloHSCT, and once a year thereafter.

All patients will be followed until 5 years after registration.

10.2 Required investigations

Overview of required minimum clinical and laboratory evaluations at registration, at entry, during treatment, and during follow up.

	Before alloHSCT	After alloHSCT (± day 28)	After PNB/DAC cycle 1 (± day 56)	After PNB/ DAC cycle 2 (± day 90)	After PNB/DAC cycle 3 (± day 139)	After PNB/DAC cycle 4 (± day 180)	9, 12,18 months after alloHSCT	2, 3, 4, 5 years after alloHSCT
Medical history	X	X	X	X	X	X	X	X
Physical exam.	X	X	X	X	X	X	X	X
Grading of GvHD		X	X	X	X	X	X	X
Hematology	X	X	X	X	X	X	X	X
Blood chemistry	X	X	X	X	X	X	X	X
BM aspirate	X	X	X	X	X	X	X	X
BM cytogenetics	X		(X)	X	(X)	X	(X)	
BM flowcytometry	X		X	X	X	X	X	
BM/PB chimerism			X	X	X	X	X	
BM/PB biobanking*	X		X	X	X	X		
Specific investigations	X**							
Pregnancy test***	X			X		X		
Response evaluation	X	X	X	X	X	X	X	X

* 20 ml BM will be used for MRD study (immunophenotyping and molecular MRD), 10 ml EDTA PB

** Pre-transplantation investigations according to local protocols (ECG, X-thorax, viral serology etc.)

*** For women of childbearing potential

(X) optional

PNB (Panobinostat); DAC (Decitabine)

Medical history

Standard medical history, with special attention for WHO performance status, infections, bleeding tendency

Only at entry: occupational history, prior and present other diseases, antecedent hematological or oncological diseases, including non-melanoma skin cancer, previous chemotherapy or radiotherapy, ethnicity

Physical examination

Standard physical examination including body weight and height, infections, bleeding tendency

Hematology

Hemoglobin, leukocyte count + differential count, ANC, platelets

Blood chemistry

Creatinine, liver enzymes, total bilirubin, albumin, LDH

Chimerism

Chimerism of bone marrow mononuclear cells, peripheral blood mononuclear cells as in table
T cell chimerism according to local practice

Grading of GvHD

Acute and chronic Graft versus Host Disease will be scored according to the criteria defined in appendix I

10.3 Response evaluation

Response will be evaluated as indicated in the table, according to appendix E

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
- No compliance of the patient

- Refusal to continue protocol treatment
- Treatment cannot be given according to the timelines described in 9.3.2 and 9.4.2

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 fulfill 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, and must be reported as described in 12.3.1

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 dose of any drug from the protocol treatment schedule 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 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 (appendix G).
Pre-existing conditions will be collected on the baseline concomitant diseases CRF, i.e. active (symptomatic) diseases of CTCAE grade ≥ 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
- Relapse/Progression of the disease under study; complications as a result of disease progression remain reportable Adverse Events
- Alopecia, nausea, vomiting, and hematological side effects

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.
Follow up information for grade 3 or 4 adverse events considered at least possibly related to the investigational medicinal product by the investigator should be reported on the AE CRF until recovery or until 6 months after the last dose of IMP, whichever comes first.
Follow up information for all other adverse events should be reported on the AE CRF until recovery or until 30 days after the last dose of any drug from the protocol treatment schedule, whichever comes first.

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 dose of any drug from the protocol treatment schedule 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.

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:

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

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, *and the manufacturer of the investigational medicinal product(s).*
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.

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), the manufacturer 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 or the manufacturer.
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 and Safety 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. 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 14.5)
- 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

Part I

- Feasibility of protocol treatment as defined by the number of DLTs during the first cycle PNB/DAC (for DLT definition see section 7.1).

Part II

- Feasibility of protocol treatment as defined by percentage of patients actually receiving treatment according to protocol up to eligibility for the first DLI within 115 days.

13.2 Secondary endpoints

- Response to first cycle PNB/DAC
- Response to second cycle PNB/DAC
- Percentage of successful donor searches
- Percentage of patients who received alloHSCT
- Best response on protocol
- Engraftment after alloHSCT
- Incidence and severity of acute and chronic GvHD
- (Serious) adverse events
- Overall survival (OS) from registration and start of protocol treatment
- PFS from registration and from start of protocol treatment
- NRM rate

14 Statistical considerations

14.1 Part I dose selection

During part I of the trial the recommended dose level (RDL) for PNB/DAC will be determined. For the dose escalation/reduction scheme, a modification of the standard 3+3-scheme is used. Based on the expected number of DLTs and the fact that 3 dose levels are considered, the decision of feasibility is based on 5+5 patients (see figure in chapter 7). For a decision to escalate to a higher dose level 10 patients have to be treated, no early decision on escalation is allowed.

A dose level is considered as not feasible if

- 3 or more DLT are observed among the first 5 patients treated at a dose level (and evaluable for DLT)

- 4 or more DLT are observed among the first 10 patients treated at a dose level (and evaluable for DLT)

In this case, an interim analysis report will be made as soon as possible and sent to the DSMB. Until a decision has been made, the study will be temporarily closed for treatment with PNB/DAC.

If the decision rules allow for dose escalation, an interim analysis report will be prepared and sent to the DMSB. The study will immediately continue at the next dose level upon consent of the DSMB. If a decision to continue to the phase II part of the trial can be made, a report of the interim analysis is sent to the DSMB and subsequently discussed within the writing committee with the DSMB's advice. Until a decision to continue to the phase II part is made, the study will remain open. The study will continue as Phase II after a decision has been made by the principal investigator taken into account the advice of the DSMB.

The characteristics of the decision rules described above have been evaluated with 10.000 simulations (each simulation representing a trial at a certain dose level).

True probability of DLT	Percentage of dose levels not considered feasible after 5 patients	Percentage of dose levels not considered feasible
10%	1%	2%
20 %	6%	14%
30 %	17%	37%
40 %	33%	64%
50 %	50%	83%

So, if the true probability of DLT is 40%, then in 64% of the trials this dose level is considered not feasible according to the decision rules on the number of DLTs given above. In 33% of the trials the dose level will be considered not feasible already after the first cohort of 5 patients.

During the Part I of the study, the incidence of aGvHD is closely monitored. Besides the interim analyses on the incidence of DLT, an interim analysis focussing on the incidence of aGvHD and on NRM is performed 1 month after the last patient of a first cohort of 10 patients eligible for protocol treatment has been included.

The interim analysis will be performed earlier in case:

- 3 or more out of the first 5 patients experience aGvHD grade III/IV within 1 month after alloHSCT
- 5 or more out of the first 10 patients experience aGvHD grade III/IV within 1 month after alloHSCT

In that case, an interim analysis report will be prepared and sent to the DSMB as soon as possible and the study will be temporarily closed until a decision has been made.

14.2 Part II: Feasibility

The primary endpoint of the phase II part of the study is the percentage of patients who completed treatment according to protocol until first DLI within 115 days after alloHSCT.
Patient counts as a failure if patient
- did not receive alloHSCT, 2 cycles PNB/DAC and DLI before day 115 for whatever reason
- did not start the second cycle of PNB/DAC before day 100
- do not fulfill all eligibility criteria (see section 9.3) at start cycle I/II
- do not fulfill elibility criteria (see section 9.4.1) for first DLI.

For the Part II of the study a Simon 2-stage design is used.

- Let P_0 be the largest probability of 'successes' which, if true, implies that the feasibility is too low and therefore the present HOVON-116 schedule does not warrant further investigation. In the present trial, P_0 has been taken as 50%.
- Let P_1 be the lowest probability of 'successes' which, if true, implies that the feasibility is sufficiently high and therefore the HOVON-116 schedule does warrant further investigation in clinical trials. In the present trial, P_1 has been taken as 70%.
- Let α be the accepted probability of recommending for further investigation a regimen with true 'success' rate equal to or lower than P_0. In the present study, α has been taken as 0.10.
- Let β be the accepted probability of rejecting from further trials a regimen with the true 'success' rate at least P_1. In the present trial, β has been taken as 0.10.

The required number of patients eligible for alloHSCT is 45, with an interim analysis after 21 patients evaluable for the primary endpoint.

- If after the first 21 patients ≤ 11(52.4%) successes are observed, the trial will be closed with the conclusion that the regimen is not feasible, and should not be further investigated. Otherwise entry will be extended to 45 patients.
- If after 45 patients ≤ 26 (57.8%, 90% CI = 44.5-70.3%; 90% CI because α = 0.10) successes are observed, the conclusion will be that the regimen is not feasible, and should not be further investigated.
- Otherwise, the trial will conclude that the regimen is feasible, and warrants further investigation in this patient population.

14.3 Patient numbers and power considerations

In part I of the study, at least 10 patients evaluable for DLT are included at each dose level. Since the study remains open until a decision on the feasibility of each dose level can be made, probably a few more patients will be treated at each dose level. We remark that patients treated at the RDL can be included in the analyses of phase II part of the study as well. Therefore, we expect to include 60 -70 patients who start with protocol treatment. From the patients registered in the study about 30-40% is expected not be eligible to start protocol treatment, due to non-availability of a donor (approximately 30%) or not reaching CR/PR (approximately 10%) after induction cycle II. So, 100-110patients will be registered in the trial.

14.4 Statistical analysis

In part II of the study only patients who started with PNB/DAC are considered evaluable of DLT. All other analyses are performed according to the intention to treat principle restricted to patients eligible for registration or to patients eligible to start protocol treatment (depending on the endpoint considered).

14.4.1 Feasibility

The main endpoint of part II of the trial is feasibility defined as the percentage of patients completing protocol treatment until first DLI within 115 days. The feasibility rate will be calculated together with the 95% confidence interval.
Also, the number and percentage of successful donor searches is reported.

14.4.2 Efficacy analysis

For the registered patients, the actuarial estimations for OS and PFS computed from registration are analysed. Restricted to the patients eligible to start protocol treatment OS and PFS are analysed computed from start alloHSCT.

14.4.3 Toxicity analysis

Per dose level, the analysis of (serious) adverse events will be primarily done by tabulation of the incidence and nature of adverse events by treatment cycle. Special attention is given to incidence and severity of acute and chronic GvHD post alloHSCT.

14.4.4 Statistical analysis plan

A detailed statistical analysis plan (SAP) will be made before the final analysis. This SAP will be discussed with the principal investigator. However this will only affect the exploratory analyses, the analysis of the primary endpoint on which the sample size calculation is based, will not be changed.

14.5 Interim analyses

All interim analysis reports will be sent to the DSMB as well as the principal investigator.

14.5.1 Part I

In part I interim analyses are performed if the decision rules imply dose escalation, dose reduction, stopping, or continuing as Phase II.

An additional interim analysis focusing on the incidence of aGvHD and NRM is performed 1 month after the first cohort of 10 patients eligible for protocol treatment is included in the study.

14.5.2 Part II

One interim analysis is planned during part II of the study as described above.
The report of this analysis will be made after the first 21 patients started with protocol treatment are evaluable up to first DLI. In this interim analysis report, next to general information as well as data on the feasibility, attention will be given to (acute and chronic) GvHD and NRM of the patients during the entire protocol treatment time (as far as these data are available).

15 Registration

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

Eligible patients should be registered before start of treatment. Patients need to be registered 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
- Local patient code (optional)
- Sex
- Date of birth
- Date written informed consent
- Date of diagnosis
- Cytogenetic risk classification
- Registration in other HOVON trial
- Planned date induction cycle II
- Specific items patient gives consent for (see ICF)
- Eligibility criteria

All eligibility criteria will be checked with a checklist.
Each patient will be given a unique patient study number (a sequence number by order of enrolment in the trial). Patient study number will be given immediately by TOP or phone and confirmed by fax or email.

Eligibility for alloHSCT should also be registered at HOVON Data Center, **regardless if the patient is eligible for alloHSCT or not,** by phone or by faxing the alloHSCT eligibility form (2). During part I of the trial the drug combination and dose level for decitabine will be assigned for eligible patients at that moment.

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.

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. 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.

16.1.1 DLT data reporting

To monitor the incidence of dose limiting toxicity (DLT) a separate CRF (DLT-form) will be used. This DLT-form must be filled out by the responsible investigator for every patient, during the phase I part of the trial between start cycle 1 PNB/DAC and start cycle 2 PNB/DAC or day 35 after start cycle 1 (whichever occurs first). The form should be dated, signed by the responsible investigator and returned to the HOVON Data Center by fax within 24 hours after DLT-occurrence, or if no DLT occurred at start cycle 2 PNB/DAC or at day 35 after start cycle 1 (whichever occurs first).

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

The content of the patient information letter, informed consent form and any other written information to be provided to patients will be in compliance with ICH-GCP and other applicable regulations and should be approved by the Ethics Committee in advance of use.
The patient information letter, informed consent form and any other written information to be provided to patients will be revised whenever important new information becomes available that may be relevant to the patient's consent. Any revised informed consent form and written information should be approved by the Ethics Committee in advance of use. The patient should be informed in a timely manner if new information becomes available that might be relevant to the patient's willingness to continue participation in the trial. The communication of this information should be documented.

17.4 Benefits and risks assessment.

Although alloHSCT is standard care in poor-risk AML, relapse after alloHSCT is high, leaving patients without curative options.
In this protocol a short-course post-transplant GvHD prophylaxis plus early post-transplant epigenetic treatment consisting of panobinostat and decitabine followed by donor lymphocyte infusions is evaluated. The aim is to prevent early relapse prior to DLI and to optimally profit from the allogeneic Graft versus Leukemia effect, to improve outcome.
The risks associated with this procedure are opportunistic infections associated with neutropenia and lymphopenia, that may occur after PNB/DAC, as compared to standard alloHSCT.

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 the last patient's last visit.

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)
Add and remove as applicable

AE	Adverse Event
AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
ATG	Anti Thymocyte Globulin
BM	Bone Marrow
Ca	Calcium
CA	Competent Authority
CALGB	Cancer and Leukemia Group B
CCR	Conventional Chemotherpay Regimen????
CHMP	Committee for Medicinal Products for Human Use
CR	Complete Remission
CRi	Complete Remission with incomplete blood count recovery
CRF	Case Report Form
CIBMTR	Center for International Blood & Marrow Transplant Research
CMML	Chronic Myelomonocytic Leukemia
CTCAE	Common Terminology Criteria for Adverse Events
DAC	Deacetylases
DAC	Decitabine
DFS	Disease Free Survival
DLI	Donor Lymphocyte infusion
DLT	Dose limiting toxicity
DNMT	DNA-methyltransferases
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
EBMT	European Group for Blood and Marrow Transplantation
EFS	Event Free Survival
EVI-1	Ecotropic viral integration site 1
EMEA	European Medicines Agency
FAB	French American British
FDA	Federal Drug Administration
FFS	Failure Free Survival
FISH	Fluorescence In Situ Hybridisation
GCP	Good Clinical Practice
G-CSF	Granulocyte-Colony Stimulating Factor

GI	Gastro-intestinal
GvHD	Graft Versus Host Disease
GVL	Graft Versus Leukemia
HAT	Histone Acetyltransferase
Hb	Hemoglobin
HDACi	Histone Deacetylase inhibitors
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte histocompatibility Antigen
HOVON	Dutch-Belgian Hematology-Oncology Cooperative Group
HRC	Hematocytology Review Committee
HSCT	Hematopoietic Stem Cell Transplantation
ICH	International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use
IMP	Investigational Medicinal Product
IPSS	International Prognostic Scoring System
ITT	Intention To Treat
IU	International Units
KCl	Potassium chloride
LDH	Lactate Dehydrogenase
MA	Myelo Ablative
MDS	Myelo Dysplastic Syndrome
METC	Medical Ethical Review Committee
MK	Monosomal Karyotype
MRD	Minimal Residual Disease
MTD	Maximum Tolerated Dose
NaCl	Sodium Chloride
NCI	National Cancer Institute
NRM	Non-Relapse Mortality
NYHA	New York Heart Association
OS	Overall Survival
PB	Peripheral Blood
PBMC	Peripheral Blood Mononuclear Cell
PD	Progressive Disease
PET	Positron Emission Tomografy
PFS	Progression Free Survival
PNB	Panobinostat
PO	Per Os

PR	Partial Response
RAEB(-T)	Refractory Anemia with Excess Blasts (in Transformation)
RIC	Reduced Intensity Conditioning
QoL	Quality of Life
SAE	Serious Adverse Event
SC	Subcutaneous
SCT	Stem Cell Transplantation
SD	Stable Disease
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMA	Tissue Micro Array
TRM	Treatment Related Mortallity
ULN	Upper Limit of Normal
WHO	World Health Organization
WMO	Wet Medisch-Wetenschappelijk Onderzoek met mensen

19 References

1. Dohner, H., et al., *Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet.* Blood, 2010. **115**(3): p. 453-74.
2. Cornelissen, J.J., et al., *Results of a HOVON/SAKK donor versus no-donor analysis of myeloablative HLA-identical sibling stem cell transplantation in first remission acute myeloid leukemia in young and middle-aged adults: benefits for whom?* Blood, 2007. **109**(9): p. 3658-66.
3. Koreth, J., et al., *Allogeneic stem cell transplantation for acute myeloid leukemia in first complete remission: systematic review and meta-analysis of prospective clinical trials.* JAMA : the journal of the American Medical Association, 2009. **301**(22): p. 2349-61.
4. Appelbaum, F.R., *Hematopoietic cell transplantation from unrelated donors for treatment of patients with acute myeloid leukemia in first complete remission.* Best practice & research. Clinical haematology, 2007. **20**(1): p. 67-75.
5. Breems, D.A. and B. Lowenberg, *Acute myeloid leukemia with monosomal karyotype at the far end of the unfavorable prognostic spectrum.* Haematologica, 2011. **96**(4): p. 491-3.
6. Cornelissen, J.J., et al., *Comparative analysis of the value of allogeneic hematopoietic stem-cell transplantation in acute myeloid leukemia with monosomal karyotype versus other cytogenetic risk categories.* J Clin Oncol, 2012. **30**(17): p. 2140-6.
7. Armistead, P.M., et al., *Quantifying the survival benefit for allogeneic hematopoietic stem cell transplantation in relapsed acute myelogenous leukemia.* Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 2009. **15**(11): p. 1431-8.
8. Schmid, C., et al., *Long-term survival in refractory acute myeloid leukemia after sequential treatment with chemotherapy and reduced-intensity conditioning for allogeneic stem cell transplantation.* Blood, 2006. **108**(3): p. 1092-9.
9. Schmid, C., et al., *Early allo-SCT for AML with a complex aberrant karyotype--results from a prospective pilot study.* Bone marrow transplantation, 2012. **47**(1): p. 46-53.
10. de Lima, M., et al., *Maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation for recurrent acute myelogenous leukemia or myelodysplastic syndrome: a dose and schedule finding study.* Cancer, 2010. **116**(23): p. 5420-31.
11. Goodyear, O., et al., *Induction of a CD8+ T-cell response to the MAGE cancer testis antigen by combined treatment with azacitidine and sodium valproate in patients with acute myeloid leukemia and myelodysplasia.* Blood, 2010. **116**(11): p. 1908-18.
12. Rothenberg, M.L., et al., *Mortality associated with irinotecan plus bolus fluorouracil/leucovorin: summary findings of an independent panel.* J Clin Oncol, 2001. **19**(18): p. 3801-7.
13. Rudek, M.A., et al., *Pharmacokinetics of 5-azacitidine administered with phenylbutyrate in patients with refractory solid tumors or hematologic malignancies.* J Clin Oncol, 2005. **23**(17): p. 3906-11.
14. Baron, F. and R. Storb, *Hematopoietic cell transplantation after reduced-intensity conditioning for older adults with acute myeloid leukemia in complete remission.* Current opinion in hematology, 2007. **14**(2): p. 145-51.
15. Blaise, D., et al., *Current status of reduced-intensity-conditioning allogeneic stem cell transplantation for acute myeloid leukemia.* Haematologica, 2007. **92**(4): p. 533-41.
16. Horwitz, M.E., *Reduced intensity versus myeloablative allogeneic stem cell transplantation for the treatment of acute myeloid leukemia, myelodysplastic syndrome and acute lymphoid leukemia.* Current opinion in oncology, 2011. **23**(2): p. 197-202.
17. Luger, S.M., et al., *Similar outcomes using myeloablative vs reduced-intensity allogeneic transplant preparative regimens for AML or MDS.* Bone marrow transplantation, 2012. **47**(2): p. 203-11.
18. Valcarcel, D., et al., *Sustained remissions of high-risk acute myeloid leukemia and myelodysplastic syndrome after reduced-intensity conditioning allogeneic hematopoietic transplantation: chronic graft-versus-host disease is the strongest factor improving survival.* J Clin Oncol, 2008. **26**(4): p. 577-84.
19. Hegenbart, U., et al., *Treatment for acute myelogenous leukemia by low-dose, total-body, irradiation-based conditioning and hematopoietic cell transplantation from related and unrelated donors.* J Clin Oncol, 2006. **24**(3): p. 444-53.
20. Jan J Cornelissen, A.G., Kees GM van Montfort, Thomas Pabst, Johan Maertens, H. Berna Beverloo, Marinus van Marwijk Kooy, P.W. Wijermans, Bart J Biemond, Dimitri A. Breems, Edo Vellenga, Leo F Verdonck, Martin F. Fey, Mojca Lavrencic, Jeroen JWM Janssen, Gerwin Huls, Jurgen Kuball, Jakob R. Passweg, Carlos Graux, Harry C. Schouten, Gert Ossenkoppele, and Bob Lowenberg, *Allogeneic Hematopoietic Stem Cell Transplantation (alloHSCT) Improves Outcome As Compared to Conventional Consolidation in Patients Aged 40–60 Years with AML in CR1 with Apparent Greater Benefit for Reduced Intensity Rather Than Myeloablative Conditioning.* Blood (ASH Annual Meeting Abstracts), Nov 2011; 118: 159, 2011. **118**.
21. Martin Bornhauser, J.K., Rudolf Trenschel, Andreas Burchert, Ute Hegenbart, Michael Stadler, Herrad Baurmann, K. Schäfer-Eckart, Ernst Holler, Nicolaus Kröger, Christoph Schmid, Hermann Einsele, Michael Kiehl, Rainer Schwerdtfeger, Arnold Ganser, Peter Dreger, Andreas Neubauer, Wolfgang E. Berdel, Gerhard Ehninger, Dietrich Beelen, Johannes Schetelig, and Matthias Stelljes, *An Intergroup Randomised Trial of Standard Intensity Versus Reduced Intensity TBI-Based Conditioning In Patients with Acute Myeloid Leukemia in First Complete Remission.* Blood (ASH Annual Meeting Abstracts), 2011. **118**.
22. Mohty, M., et al., *Reduced intensity conditioning allogeneic stem cell transplantation for patients with acute myeloid leukemia: long term results of a 'donor' versus 'no donor' comparison.* Leukemia, 2009. **23**(1): p. 194-6.

23. Farag, S.S., et al., *Comparison of reduced-intensity hematopoietic cell transplantation with chemotherapy in patients age 60-70 years with acute myelogenous leukemia in first remission.* Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 2011. **17**(12): p. 1796-803.
24. Burroughs, L., et al., *Extending postgrafting cyclosporine decreases the risk of severe graft-versus-host disease after nonmyeloablative hematopoietic cell transplantation.* Transplantation, 2006. **81**(6): p. 818-25.
25. Maris, M.B., et al., *HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies.* Blood, 2003. **102**(6): p. 2021-30.
26. Maris, M.B., et al., *Unrelated donor granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing.* Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 2006. **12**(4): p. 454-65.
27. McSweeney, P.A., et al., *Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects.* Blood, 2001. **97**(11): p. 3390-400.
28. Niederwieser, D., et al., *Low-dose total body irradiation (TBI) and fludarabine followed by hematopoietic cell transplantation (HCT) from HLA-matched or mismatched unrelated donors and postgrafting immunosuppression with cyclosporine and mycophenolate mofetil (MMF) can induce durable complete chimerism and sustained remissions in patients with hematological diseases.* Blood, 2003. **101**(4): p. 1620-9.
29. Brunstein, C.G., et al., *Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts.* Blood, 2011. **118**(2): p. 282-8.
30. Luznik, L., et al., *High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease.* Blood, 2010. **115**(16): p. 3224-30.
31. Raiola, A.M., et al., *Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning.* Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 2013. **19**(1): p. 117-22.
32. Luznik, L., et al., *HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide.* Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 2008. **14**(6): p. 641-50.
33. Ciurea, S.O., et al., *Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation.* Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation, 2012. **18**(12): p. 1835-44.
34. Emadi, A., R.J. Jones, and R.A. Brodsky, *Cyclophosphamide and cancer: golden anniversary.* Nature reviews. Clinical oncology, 2009. **6**(11): p. 638-47.
35. Lubbert, M. and M. Minden, *Decitabine in acute myeloid leukemia.* Seminars in hematology, 2005. **42**(3 Suppl 2): p. S38-42.
36. Kantarjian, H., et al., *Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia.* Blood, 2007. **109**(1): p. 52-7.
37. Lubbert, M., *DNA methylation inhibitors in the treatment of leukemias, myelodysplastic syndromes and hemoglobinopathies: clinical results and possible mechanisms of action.* Curr Top Microbiol Immunol, 2000. **249**: p. 135-64.
38. Issa, J.P., et al., *Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies.* Blood, 2004. **103**(5): p. 1635-40.
39. Zagonel, V., et al., *5-Aza-2'-deoxycytidine (Decitabine) induces trilineage response in unfavourable myelodysplastic syndromes.* Leukemia, 1993. **7 Suppl 1**: p. 30-5.
40. Wijermans, P.W., et al., *Continuous infusion of low-dose 5-Aza-2'-deoxycytidine in elderly patients with high-risk myelodysplastic syndrome.* Leukemia, 1997. **11**(1): p. 1-5.
41. Yang, A.S., et al., *DNA methylation changes after 5-aza-2'-deoxycytidine therapy in patients with leukemia.* Cancer Res, 2006. **66**(10): p. 5495-503.
42. Kantarjian, H., et al., *Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study.* Cancer, 2006. **106**(8): p. 1794-803.
43. Lubbert, M., et al., *Low-dose decitabine versus best supportive care in elderly patients with intermediate- or high-risk myelodysplastic syndrome (MDS) ineligible for intensive chemotherapy: final results of the randomized phase III study of the European Organisation for Research and Treatment of Cancer Leukemia Group and the German MDS Study Group.* J Clin Oncol, 2011. **29**(15): p. 1987-96.
44. Cashen, A.F., et al., *Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia.* J Clin Oncol, 2010. **28**(4): p. 556-61.
45. Kantarjian, H.M., et al., *Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia.* J Clin Oncol, 2012. **30**(21): p. 2670-7.
46. De Padua Silva, L., et al., *Feasibility of allo-SCT after hypomethylating therapy with decitabine for myelodysplastic syndrome.* Bone marrow transplantation, 2009. **43**(11): p. 839-43.
47. Lubbert, M., et al., *Non-intensive treatment with low-dose 5-aza-2'-deoxycytidine (DAC) prior to allogeneic blood SCT of older MDS/AML patients.* Bone marrow transplantation, 2009. **44**(9): p. 585-8.
48. Wijermans, P., et al., *Low-dose 5-aza-2'-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients.* J Clin Oncol, 2000. **18**(5): p. 956-62.
49. Ruter, B., et al., *Preferential cytogenetic response to continuous intravenous low-dose decitabine (DAC) administration in myelodysplastic syndrome with monosomy 7.* Blood, 2007. **110**(3): p. 1080-2; author reply 1083.
50. Bertz, H., K. Potthoff, and J. Finke, *Allogeneic stem-cell transplantation from related and unrelated donors in older patients with myeloid leukemia.* J Clin Oncol, 2003. **21**(8): p. 1480-4.

51. Appelbaum, F.R., et al., *End points to establish the efficacy of new agents in the treatment of acute leukemia.* Blood, 2007. **109**(5): p. 1810-6.
52. Marks, R., et al., *Reduced-toxicity conditioning with fludarabine, BCNU, and melphalan in allogeneic hematopoietic cell transplantation: particular activity against advanced hematologic malignancies.* Blood, 2008. **112**(2): p. 415-25.
53. Kim, D.Y., et al., *Feasibility of hypomethylating agents followed by allogeneic hematopoietic cell transplantation in patients with myelodysplastic syndrome.* Bone marrow transplantation, 2012. **47**(3): p. 374-9.
54. Baylin, S.B. and P.A. Jones, *A decade of exploring the cancer epigenome - biological and translational implications.* Nat Rev Cancer, 2011. **11**(10): p. 726-34.
55. Jones, P.A. and S.B. Baylin, *The epigenomics of cancer.* Cell, 2007. **128**(4): p. 683-92.
56. de Lima, M., et al., *Long-term follow-up of a phase I study of high-dose decitabine, busulfan, and cyclophosphamide plus allogeneic transplantation for the treatment of patients with leukemias.* Cancer, 2003. **97**(5): p. 1242-7.
57. Willemze, R., et al., *A randomized phase II study on the effects of 5-Aza-2'-deoxycytidine combined with either amsacrine or idarubicin in patients with relapsed acute leukemia: an EORTC Leukemia Cooperative Group phase II study (06893).* Leukemia, 1997. **11 Suppl 1**: p. S24-7.
58. Schwartsmann, G., et al., *Decitabine (5-Aza-2'-deoxycytidine; DAC) plus daunorubicin as a first line treatment in patients with acute myeloid leukemia: preliminary observations.* Leukemia, 1997. **11 Suppl 1**: p. S28-31.
59. Blum, W., et al., *Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia.* J Clin Oncol, 2007. **25**(25): p. 3884-91.
60. Garcia-Manero, G. and H.I. Saba, *Decitabine in myelodysplastic syndromes: viewpoints.* Drugs, 2006. **66**(7): p. 959-60.
61. Oki, Y., et al., *Induction of hypomethylation and molecular response after decitabine therapy in patients with chronic myelomonocytic leukemia.* Blood, 2008. **111**(4): p. 2382-4.
62. Richel, D.J., et al., *The antileukaemic activity of 5-Aza-2 deoxycytidine (Aza-dC) in patients with relapsed and resistant leukaemia.* Br J Cancer, 1991. **64**(1): p. 144-8.
63. Yee KWL, M.M., Brandwein J,, *A phase I trial of two sequence-specific schedules of decitabine and vorinostat in patients with acute myeloid leukemia (AML).* Blood, 2007. **110**.
64. Issa, J.P., Castoro R, Ravandi-Kashani F, *Randomized phase II study of combined epigenetic therapy: decitabine vs. decitabine and valproic acid in MDS and AML.* Blood, 2008. **112**.
65. Kirschbaum M, G.I., Goldberg SL, *Vorinostat in Combination with Decitabine for the Treatment of Relapsed or Newly Diagnosed Acute Myelogenous Leukemia (AML) or Myelodysplastic Syndrome (MDS): A Phase I, Dose-Escalation Study.* Blood, 2009. **114**.
66. Döhner H et al, *Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet.* Blood 2010; **115**: 453-574
67. Cheson BD et al, *Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia.* J Clin Oncol 2003; **15**: 4642-464

A. WHO Classification for acute myeloid leukemias and myelodysplastic syndromes

WHO 2008 classification for Acute Myeloid Leukemias (AML) and related precursor neoplasms

- Definition AML: ≥ 20% myeloblasts in blood or bone marrow
- Abnormal promyelocytes in acute promyelocytic leukemia, promonocytes in AML with monocytic differentiation and megakaryoblasts in acute megakaryoblastic leukemia are considered blast equivalents

WHO code	Category	Subcategory and short description
9896	AML with recurrent genetic abnormalities	AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1**
9871		AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFß-MYH11**
9866		Acute promyelocytic leukemia; AML with t(15;17)(q22;q12); *PML-RARA* and cytogenetic variants
9897		AML with t(9;11)(p22;q23); *MLLT3-MLL*
9865		AML with t(6;9)(p23;q34);*DEK-NUP214*
9869		AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
9911		AML (megakaryoblastic) with t(1;22)(p13;q13);*RBM15-MKL1*
9861		Provisional entity: AML with mutated *NPM1*
9861		Provisional entity: AML with mutated *CEBPA*
9895	AML with myelodysplasia related changes	Previous history of myelodysplastic syndrome **or** Myelodysplastic syndrome-related cytogenetic abnormality **or** Dysplasia present in > 50% of 2 or more cell lineages **Absence of both** Prior cytotoxic therapy for an unrelated disease Recurring cytogenetic abnormality as described in AML with recurrent genetic abnormalities
9920	Therapy-related myeloid neoplasms	Includes t-AML, t-MDS and t-MDS/MPN
9861	**Acute myeloid leukemia, NOS**	
9872	AML with minimal differentiation	**<**3% of blasts positive for Sudan Black B or MPO. Blasts usually express CD13 and/or CD117, with or without CD33 in absence of lymphoid markers cCD3, cCD22 and cCD79a
9873	AML without maturation	Blasts ≥90% of bone marrow non-erythroid cells (i.e. excluding also lymphocytes, plasmacells, macrophages and mast cells) ≥3% of blasts positive for Sudan Black B or MPO Blasts express MPO and one or more of myeloid-associated antigens such as CD13, CD33 or CD117
9874	AML with maturation	≥10% maturing cells of neutrophil lineage <20% bone marrow monocytes
9867	Acute myelomonocytic leukemia	**>**20% neutrophils and precursors of marrow cells **>**20% monocytes and precursors of marrow cells
9891	Acute monoblastic and monocytic leukemia	≥80% of the leukemic cells are monoblasts, promonocytes and monocytes
9840	Acute erytroid leukemia	Erythroleukemia (erythroid/myeloid) Presence of medium to large size erythroblasts: ≥ 50% of bone marrow cells Blasts: ≥ 20% of the bone marrow nonerythroid cells
		Pure erythroid leukemia Presence of medium to large size erythroblasts

9910	Acute megakaryoblastic leukemia	>50% of the blasts are of megakaryocytic lineage Blasts express CD41 and/or CD61
9870	Acute basophilic leukemia	Primary differentiation to basophils; mature basophils are usually sparse
9931	Acute panmyelosis with myelofibrosis	Acute panmyeloid proliferation with accompanying fibrosis
9930	Myeloid sarcoma	Tumor mass of myeloblasts or immature myeloid cells occurring in an anatomical site other than the bone marrow
Myeloid proliferations related to Down syndrome (DS)		
9898	Transient abnormal myelopoiesis (TAM)	Morphologic and immunophemotypic features are similar to the blasts in most cases of DS AML
9898	Myeloid leukemia associated with Down syndrome	Usually an acute megakaryoblastic leukemia
9727	**Blastic plasmacytoid dendritic cell neoplasm (BPDC)**	
		Blastic NK-cell lymphoma

*Rare cases show < 20% myeloblasts; these should be classified as AML

WHO 2008 classification for myelodysplastic syndromes

WHO code	Disease	Blood findings	Bone marrow findings
9980 *9991* *9992*	Refractory cytopenias with unilineage dysplasia (RCUD) Refractory anemia (RA); Refractory neutropenia (RN); Refractory thrombocytopenia (RT)	Unicytopenia or bicytopenia[1] No or rare blasts (<1%)[2]	Unilineage dysplasia: ≥ 10% of the cells in one myeloid lineage <5% blasts <15% of erythroid precursors are ring sideroblasts
9982	Refractory anemia with ring sideroblasts (RARS)	Anemia No blasts	≥15% of erythroid precursors are ring sideroblasts Erythroid dysplasia only <5% blasts
9985	Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenia(s) No or rare blasts (<1%)[2] No Auer rods <1 x 10^9/l monocytes	Dysplasia in ≥10% of the cells in ≥ two myeloid lineages (neutrophil and/or erythroid precursors and/or megakaryocytes) <5% blasts in marrow No Auer Rods ±15% ring sideroblasts
9983	Refractory anemia with excess blasts-1 (RAEB-1)	Cytopenia(s) <5% blasts[2] No Auer rods <1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 5-9% blasts[2] No Auer rods
9983	Refractory anemia with excess blasts-1 (RAEB-2)	Cytopenia(s) 5-19% blast Auer rods ± <1 x 10^9/L monocytes	Unilineage or multilineage dysplasia 10-19% blasts[2] Auer rods ±
9989	Myelodysplastic syndrome-unclassified (MDS-U)	Cytopenias ≤1% blasts[2]	Unequivocal dysplasia in less than 10% of the cells in one or more myeloid cell lines when accompanied by a cytogenetic abnormality considered as presumptive evidence for a diagnosis of MDS < 5% blasts
9986	MDS associated with isolated del(5q)	Anemia Usually normal or increased platelet count	Normal to increased megakaryocytes with hypolobated nuclei < 5% blasts

		No or rare blasts (<1%)	No Auer rods Isolated del(5q) cytogenetic abnormality
9985	Refractory cytopenia of childhood		

[1]Bicytopenia may occasionally be observed. Cases with pancytopenia should be classified as MDS-U
[2]If the marrow myeloblast percentage is <5% but there are 2-4% myeloblasts in the blood, the diagnostic classification is RAEB 1. Cases of RCUD and RCMD with 1% myeloblasts in the blood should be classified as MDS-U.
[3] Cases with Auer rods and <5% myeloblasts in the blood and <10% in the marrow should be classified as RAEB-2

WHO 2008 Acute leukemia's of ambiguous lineage

WHO code	**Category**	**Short description**
9801	Acute undifferentiated leukemia	Expresses no markers considered specific for either lymphoid or myeloid lineage
9806	MPAL, with t(9;22)(q34;q11.2); *BCR-ABL1*	
9807	MPAL, with t(v;11q23); *MLL* rearranged	
9808	MPAL, B/myeloid, NOS	
9809	MPAL, T/myeloid, NOS	
	MPAL, NOS-rare types	
	Other ambiguous lineage leukemia's	A combination of markers is expressed that does not allow classification as either AUL or MPAL

MPAL= mixed phenotype acute leukemia

Requirements for assessing more than one lineage to a single blast population (mixed phenotype)

Myeloid lineage Myeloperoxidase (flow cytometry, immunohistochemistry or cytochemistry) Or Mononcytic differentiation (at least 2 of the following: NSE, CD11c, CD14, CD64, lysozyme)
T lineage Cytoplasmic CD3 (flow cytometry with antibodies to CD3 epsilon chain; immunohistochemistry using polyclonal anti-CD3 antibody may detect CD3 zeta chain, which is not T-cell specific) Or Surface CD3 (rare in mixed phenotype acute leukemia's)
B lineage (multiple antigens required) Strong CD19 with at least 1 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10 Or Weak CD19 with at least 2 of the following strongly expressed: CD79a, cytoplasmic CD22, CD10

B. FAB classification of AML

Cytological criteria for the diagnosis of acute myeloid leukemia: French-American-British-(FAB) classification

FAB subtype	
	For all AML subtypes the following criteria apply: ♦ Blasts ≥ 30% of bone marrow nucleated cells, except for M3 ♦ ≥ 3% of blasts positive for Sudan BlackB or Myeloperoxidase, except for M0 and M7
M0	♦ < 3% of blasts positive for Sudan Black B or Myeloperoxidase ♦ at least one of the following myeloid markers present: CD13,CD33, CD15, CDw65 ♦ in absence of lymphoid markers CD3 and CD22
M1	♦ Blasts ≥ 90% of bone marrow nonerythroid cells (i.e. excluding also lymphocytes, plasma cells, macrophages and mast cells) ♦ Maturing granulocytic cells (i.e. promyelocytes towards polymorphonuclear cells ≤ 10% of nonerythroid cells ♦ (pro)monocytes ≤ 10% of nonerythroid marrow cells
M2	♦ Blasts 30-89% of bone marrow nonerythroid cells ♦ Maturing granulocytic cells (i.e. promyelocytes to polymorphonuclear cells) > 10% of nonerythroid cells ♦ Monocytic cells (i.e. monoblasts to monocytes) < 20% of nonerythroid cells
M2E	♦ Analogous to M4E, but lacking clear monocytic differentiation
M3	♦ Promyelocytes (most hypergranular) > 30% of bone marrow nucleated cells
M3V	♦ Promyelocytes (hypogranular or microgranular) > 30% of bone marrow nucleated cells
M4	♦ Granulocytic cells (myeloblasts to polymorphonuclear cells) ≥ 20% of nonerythroid cells plus one of the following criteria • Monocytic cells (monoblasts to monocytes) ≥ 20% of nonerythroid cells Or • Peripheral blood monocytes ≥ 5 x 10^9/l Or • Elevated urinary lysozymes ≥ 3 x normal value
M4E	♦ Same as M4, but with ≥ 5% abnormal eosinophils (basophilic granulae)
M5A	♦ Blasts ≥ 30% of bone marrow nonerythroid cells ♦ Bone marrow monocytic component ≥ 80% of nonerythroid cells ♦ Monoblasts ≥ 80% of bone marrow monocytic component
M5B	♦ Blasts ≥ 30% of bone marrow nonerythroid cells ♦ Bone marrow monocytic component ≥ 80% of nonerythroid cells ♦ Monoblasts < 80% of bone marrow monocytic component
M6	♦ Erythroblasts ≥ 50% of bone marrow nucleated cells ♦ Blasts ≥ 30% of bone marrow nonerythroid cells
M7	♦ > 30% of bone marrow nucleated cells are megakaryoblasts CD41 or CD61 positive or ♦ Platelet specific peroxidase reaction (electron microspcopy) ♦ < 3% of blasts positive for Sudan Black B or Myeloperoxidase

C. International Prognostic Score System (IPSS) for MDS

Revised International Prognostic Score System (IPSS-R) for MDS

Risk category	IPSS-R score
Very low	≤ 1.5
Low	> 1.5-3
Intermediate	> 3-4.5
High	> 4.5-6
Very high	> 6

IPSS-R prognostic score values

Prognostic score value	0	0.5	1	1.5	2	3	4
Cytogenetic	Very good	-	Good	-	Intermediate	Poor	Very poor
BM blast, %	≤2	-	>2- <5	-	5-10	>10	-
Hemoglobin mmol/l	≥6.2	-	5.0- <6.2	<5.0	-	-	-
Platelets $x10^9$/l	≥100	50- 100	<50	-	-	-	-
ANC $x10^9$/l	≥0.8	<0.8	-	-	-	-	-

- indicates 'not applicable'

Cytogenetic scoring system for IPSS-R

Prognostic subgroups	Cytogenetic abnormalities
Very good	-Y,del(11q)
Good	Normal, del(5q),del(12p),del(20q), double including del(5q)
Intermediate	del(7q),+8,+19,i(17q), any other single or double independent clones
Poor	-7, inv(3)/t(3q),del(3q), double including -7/del(7q), complex: 3 abnormalities
Very poor	Complex: >3 abnormalities

Greenberg PL et al., Blood, 120;(12): 2454-2465

D. Prognostic relapse risk group definition

Patients are classified in 4 risk groups according to the table below.

Risk		Definition	% pts at baseline	% pts with CR & consolidation
Good	GR1	t(8;21) or *AML1-ETO*, WBC≤20	5 %	7 %
	GR2	inv(16)/t(16;16) or *CBFB-MYH11* gene	6 %	7 %
	GR3	MK-, *CEBPA*+	7 %	8 %
	GR4	MK-, *FLT3ITD-/NPM1+,* CRe	11 %	13 %
Intermediate	IR1	t(8;21) or *AML1-ETO*, WBC>20	2 %	2 %
	IR2	CN –X –Y, WBC≤100, CRe	17 %	21 %
Poor	PR1	CN –X –Y, WBC≤100, not CRe	10 %	8 %
	PR2	CN –X –Y, WBC>100	5 %	4 %
	PR3	CA, non CBF, MK-, no abn3q26, EVI1-	16 %	15 %
Very Poor	VPR1	Non CBF, MK+	9 %	5 %
	VPR2	Non CBF, abn3q26	2 %	1 %
	VPR3	Non CBF, EVI1+	9 %	9 %

The table gives the % distribution of each risk subgroup of all patients at diagnosis and of all patients that have reached CR and have received consolidation treatment.

- The core-binding factor (CBF) leukemias involve AML's with cytogenetic abnormality t(8;21)(q22;q22) or the *AML1-ETO* fusion gene and the cytogenetic abnormalities inv(16)(p13q22) or t(16;16)(p13;q22) or the related fusion gene *CBFB-MYH11*.
- If cytogenetics unknown, consider as CN
- Monosomal karyotype (MK) refers to AML with two or more autosomal monosomies or a single autosomal monosomy in the presence of one or more structural cytogenetic abnormalities
- MK-: monosomal karyotype negative
- MK+: monosomal karyotype positive
- CN –X-Y: cytogenetically normal or only loss of X or Y chromosome
- CA: cytogenetically abnormal
- CRe: attainment of early CR, ie after cycle I
- EVI1+ refers to high EVI1 mRNA expression
- FLT3-ITD-/NMP1+ : FLT3-ITD mutant negative (FLT3ITD-) but NPM1-mutant positive (NPM1+): Fms-like tyrosine kinase receptor-3 internal tandem duplications (FLT3-ITD) and nucleophosmin-1 (NPM!) mutations often go together as dual genetic anomalies in the same AML.
- *To exclude ambiguities in the classification patients should be classified in the following hierarchical order: first patients with CBF abnormalities in GR1, GR2 or IR1, of the remaining patients the MK+ patients in VPR1, followed by the abn3q26 patients in VPR2 subsequently the CEBPA+ patients in GR3 and the FLT3ITD-/NPM1+ patients in GR4, subsequently the EVI1+ patients in VPR3. The remaining patients are classified in PR1, IR2, PR2 and PR3.*

Risk based in addition on marker and microarray expression data

For a smaller set of 424 patients also gene marker information and microarray expression data were available. Analysis of these data were consistent with results by others: AML with *CEBP*A mutations and AML with *FLT3ITD-/NPM1+* (ie *NPM1* mutation without FLT3-ITD mutation) have a favorable prognosis, while leukemias with high *EVI1+* mRNA expression show a very poor prognosis. Combination of the cytogenetic, the WBC , the early or late CR and the molecular information led to the extended risk classification shown at the beginning of this appendix.

A summary of the OS and EFS of the patients in the previous HOVON/SAKK AML studies is shown in the table below for each of the risk (sub)groups. The most relevant estimates are OS2 and EFS2 which are the 5 year overall survival and event free survival measured from the start of consolidation treatment and which are restricted to patients who have reached a CR on protocol after cycle I or II and who received consolidation treatment. These are the patients for which a choice must be made between consolidation with chemotherapy cycle III, an autologous transplant or an allogeneic transplant. Estimates from diagnosis have been added for completeness, although at diagnosis knowledge about the achievement of (early) complete remission is still unavailable.

Risk			From diagnosis			From start consolidation	
			CR1	EFS1	OS1	EFS2	OS2
Good			**94***	**51**	**65**	**58**	**76**
	GR1	t(8;21), WBC≤20	94	59	68	66	75
	GR2	inv(16)/t(16;16)	93	44	68	50	77
	GR3	MK-, *CEBPA*+	84	48	61	59	67
	GR4	MK-, *FLT3ITD-/NPM1*+, CRe	100*	51	57	59	61
Inter-mediate			**99***	**42**	**51**	**48**	**55**
	IR1	t(8;21), WBC>20	87	32	46	35	50
	IR2	CN –X –Y, WBC≤100, CRe	100*	43	51	48	55
Poor			**75***	**19**	**25**	**27**	**33**
	PR1	CN –X –Y, WBC≤100, not CRe CN	69*	17	23	24	31
	PR2	–X –Y, WBC>100	74*	23	27	32	37
	PR3	CA, non CBF,MK-,no abn3q26, *EVI1*-	79	20	25	27	33
Very Poor			**60**	**3**	**7**	**7**	**12**
	VPR1	Non CBF, MK+	48	2	4	6	9
	VPR2	Non CBF, abn3q26	65	8	19	8	12
	VPR3	Non CBF, *EVI1*+	79	10	17	10	16

Table gives the outcome of therapy for each of the prognostic risk subgroups as regards CR, EFS and OS (from diagnosis) or from consolidation (EFS2, OS2)

CR1 % patients reaching CR after cycle I or cycle II

EFS1 actuarial probability of event free survival 5 year from diagnosis

OS1 actuarial probability of overall survival 5 year from diagnosis

EFS2 actuarial probability of event free survival 5 year from start consolidation

OS2 actuarial probability of overall survival 5 year from start consolidation

* Note that the risk classification includes early CR (after cycle 1) or late CR as a criterion for some classes. This has an impact on the estimates from diagnosis.

E. Response criteria

These response criteria were published in the 2009 paper, "Diagnosis and management of acute myeloid leukemia in adults: recommendations from an international expert panel, on behalf of the European LeukemiaNet" [(66)], and are based on International Working Group recommendations published in 2003 [(67)].

CATEGORY	DEFINITION
Complete remission (CR) [1]	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count >1.0 x 10^9/L; platelet count >100 x 10^9/L; independence of red cell transfusions
CR with incomplete recovery (CRi) [2]	All CR criteria except for residual neutropenia (<1.0 x 10^9/L) or thrombocytopenia (<100 x 10^9/L)
Morphologic leukemia-free state [3]	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	Relevant in the setting of phase I and II clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%
Cytogenetic CR (CRc) [4]	Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow
Molecular CR (CRm) [5]	No standard definition; depends on molecular target
Resistant disease (RD)	Failure to achieve CR or CRi (general practice; phase II/III trials), or failure to achieve CR, CRi or PR (phase I trials); only includes patients surviving > 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring > 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 days following its completion; or deaths occurring > 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available
Relapse [6]	Bone marrow blasts > 5%; or reappearance of blasts in the blood; or development of extramedullary disease

[1] All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5-7 days; flow cytometric evaluation may help to distinguish between persistent leukemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

[2] The criterion of CRi is of value in protocols using intensified induction or double induction strategies, in which hematologic recovery is not awaited, but intensive therapy will be continued. In such protocols, CR may even not be achieved in the

course of the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients. Some patients may not achieve complete hematologic recovery upon longer observation times.

[3] This category may be useful in the clinical development of novel agents within phase I clinical trials, in which a transient morphologic leukemia-free state may be achieved at the time of early response assessment.

[4] Four studies showed that failure to convert to a normal karyotype at the time of CR predicts inferior outcome.

[5] As an example, in CBF AML low-level PCR-positivity can be detected in patients even in long-term remission. Normalizing to 104 copies of ABL1 in accordance with standardized criteria, transcript levels below 12 to 10 copies appear to be predictive for long-term remission.

[6] In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of hematopoiesis. Cytogenetics should be tested to distinguish true relapse from therapy-related MDS/AML.

F. ZUBROD-ECOG-WHO Performance Status Scale

0 Normal activity

1 Symptoms, but nearly ambulatory

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

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

4 Unable to get out of bed

5 Death

G. 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)

H. NYHA scoring list

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

Grade 1	No breathlessness
Grade 2	Breathlessness on severe exertion
Grade 3	Breathlessness on mild exertion
Grade 4	Breathlessness at rest

I. Diagnosis, staging and grading of GvHD

Diagnosis

Acute and chronic GvHD is defined according to the proposal of the recent National Institutes of Health (NIH) Consensus Conference, which recognizes 2 categories of GvHD [1]:

(1) acute GvHD (absence of features consistent with chronic GvHD), comprising:

(a) classic acute GvHD (before day 100), and,

(b) persistent, recurrent, or late acute GvHD (after day 100, often upon withdrawal of immunosuppression);

(2) chronic GvHD, comprising:

(a) classic chronic GvHD (no signs of acute GvHD), and,

(b) an overlap syndrome, in which features of both acute and chronic GvHD are present.

Staging and grading of acute GvHD

For staging and grading the Glucksberg classification updated according to Przepiorka et al [2,3] is used:

Stage	Skin Rash	Liver Total bilirubin (μmol/L)	Intestinal tract Diarrhea (ml/day)
1	<25%	34-50	500 –1000 or persistent nausea without diarrhea*
2	25-50%	50-102	1000-1500
3	> 50%	102-255	>1500
4	generalized erythroderma with bullous formation	>255	severe pain/ileus
Grade			
I	Skin: stage 1-2 and Liver: stage 0 and Gut: stage 0		
II	Skin: stage 3 or Liver: stage 1 or Gut: stage 1		
III	Liver: stage 2-3 or Gut: stage 2-4		
IV	Skin or Liver: stage 4		

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

Signs and symptoms of chronic GvHD according to the National Institutes of Health (NIH) Consensus Conference

Organ or Site	Diagnostic (sufficient to establish the diagnosis of chronic GvHD)	Distinctive (seen in chronic GvHD, but insufficient alone to establish the diagnosis of chronic GvHD)	Other features*	Common (seen with both acute and chronic GvHD)
Skin	Poikiloderma Lichen planus-like features Sclerotic features Morphea-like features Lichen sclerosus-like features	Depigmentation	Sweat impairment Ichthyosis Keratosis pilaris Hypopigmentation Hyperpigmentation	Erythema Maculopapular rash Pruritus
Nails		Dystrophy Longitudinal ridging, splitting, or brittle features Onycholysis Pterygium unguis Nail loss (usually symmetric; affects most nails)		
Scalp and body hair		New onset of scarring or nonscarring scalp alopecia (after recovery from chemoradiotherapy) Scaling, papulosquamous lesions	Thinning scalp hair, typically patchy, coarse, or dull (not explained by endocrine or other causes) Premature gray hair	
Mouth	Lichen-type features Hyperkeratotic plaques Restriction of mouth opening from sclerosis	Xerostomia Mucocele Mucosal atrophy Pseudomembranes Ulcers		Gingivitis Mucositis Erythema Pain
Eyes		New onset dry, gritty, or painful eyes[#] Cicatricial conjunctivitis Keratoconjunctivitis sicca Confluent areas of punctate keratopathy	Photophobia Periorbital hyperpigmentation Blepharitis (erythema of the eyelids with edema)	
Genitalia	Lichen planus-like features Vaginal scarring or stenosis	Erosions Fissures Ulcers		
GI tract	Esophageal web Strictures or stenosis in the upper to mid third of the esophagus		Exocrine pancreatic insufficiency	Anorexia Nausea Vomiting Diarrhea Weight loss Failure to thrive (infants and children)
Liver				Total bilirubin, alkaline phosphatase >2 × upper limit of normal ALT or AST >2 × upper limit of normal
Lung	Bronchiolitis obliterans diagnosed with lung biopsy	Bronchiolitis obliterans diagnosed with PFTs and radiology		BOOP

Muscles, fascia, joints	Fasciitis Joint stiffness or contractures secondary to sclerosis	Myositis or polymyositis	Edema Muscle cramps Arthralgia or arthritis	
Hematopoietic and immune			Thrombocytopenia Eosinophilia Lymphopenia Hypo- or hypergammaglobulinemia Autoantibodies (AIHA and ITP)	
Other			Pericardial or pleural effusions Ascites Peripheral neuropathy Nephrotic syndrome Myasthenia gravis Cardiac conduction abnormality or cardiomyopathy	

* Can be acknowledged as part of the chronic GvHD symptomatology if the diagnosis is confirmed
[#] Diagnosis of chronic GvHD requires biopsy or radiology confirmation (or Schirmer test for eyes).

Organ scoring of chronic GvHD

PERFORMANCE SCORE:	**SCORE 0**	**SCORE 1**	**SCORE 2**	**SCORE 3**
KPS / ECOG / LPS	Asymptomatic and fully active (ECOG 0; KPS or LPS 100%)	Symptomatic, fully ambulatory, restricted only in physically strenuous activity (ECOG 1, KPS or LPS 80-90%)	Symptomatic, ambulatory, capable of selfcare, >50% of waking hours out of bed (ECOG 2, KPS or LPS 60-70%)	Symptomatic, limited self-care, >50% of waking hours in bed (ECOG 3-4, KPS or LPS <60%)
SKIN *Clinical features:* Maculopapular rash Lichen planus-like features Papulosquamous lesions or ichthyosis Hyperpigmentation Hypopigmentation Keratosis pilaris Erythema Erythroderma Poikiloderma Sclerotic features Pruritus Hair involvement Nail involvement **% BSA involved**	No Symptoms	**<**18% BSA with disease signs but **NO** sclerotic features	19-50% BSA **OR** Involvement with superficial sclerotic features “not hidebound” (able to pinch)	>50% BSA **OR** deep sclerotic features “hidebound” (unable to pinch) **OR** impaired mobility, ulceration or severe pruritus
MOUTH	No symptoms	Mild symptoms with disease signs but not limiting oral intake significantly	Moderate symptoms with disease signs **with** partial limitation of oral intake	Severe symptoms with disease signs on examination **with** major imitation of oral intake

EYES Mean tear test (mm): >10 6-10 ≤5 Not done	No symptoms	Mild dry eye symptoms not affecting ADL (requiring eyedrops ≤ 3 x per day) **OR** asymptomatic signs of keratoconjunctivitis sicca	Moderate dry eye symptoms partially affecting ADL (requiring drops > 3 x per day or punctal plugs), **WITHOUT** vision impairment	Severe dry eye symptoms significantly affecting ADL (special eyeware to relieve pain) **OR** Unable to work because of ocular symptoms **OR** Loss of vision caused by keratoconjunctivitis sicca
GI TRACT	No symptoms	Symptoms such as dysphagia, anorexia, nausea, vomiting, bdominal pain or diarrhea without significant weight loss (<5%)	Symptoms associated with mild to moderate weight loss (5-15%)	Symptoms associated with significant weight loss >15%, requires nutritional supplement for most calorie needs **OR** esophageal dilation
LIVER	Normal LFT	Elevated Bilirubin, AP, AST or ALT <2 x ULN	Bilirubin >3 mg/dl (> 50 µmol/L) or Bilirubin, enzymes 2-5 x ULN	Bilirubin or enzymes > 5 x ULN
LUNGS†	No symptoms FEV1 > 80% **OR** LFS=2	Mild symptoms (shortness of breath after climbing one flight of steps) FEV1 60-79% **OR** LFS 3-5	Moderate symptoms (shortness of breath after walking on flat ground) FEV1 40-59% **OR** LFS 6-9	Severe symptoms (shortness of breath at rest; requiring 0_2) FEV1 ≤39% **OR** LFS 10-12
JOINTS AND FASCIA	No symptoms	Mild tightness of arms or legs, normal or mild decreased range of motion (ROM) **AND** not affecting ADL	Tightness of arms or legs **OR** joint contractures, erythema thought due to fasciitis, moderate decrease ROM **AND** mild to moderate limitation of ADL	Contractures **WITH** significant decrease of ROM **AND** significant limitation of ADL (unable to tie shoes, button shirts, dress self etc.)
GENITAL TRACT	No symptoms	Symptomatic with mild signs on exam **AND** no effect on coitus and minimal discomfort with gynecologic exam	Symptomatic with moderate signs on exam **AND** with mild dyspareunia or discomfort with gynecologic exam	Symptomatic **WITH** advanced signs (stricture, labial agglutination or severe ulceration) **AND** severe pain with coitus or inability to insert vaginal speculum

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

Esophageal stricture or web	Pericardial Effusion	Pleural Effusion(s)
Ascites (serositis)	Nephrotic syndrome	Peripheral Neuropathy
Myasthenia Gravis	Cardiomyopathy	Eosinophilia > 500µl
Polymyositis	Cardiac conduction defects	Coronary artery involvement
Platelets <100,000/µl	Progressive onset	Other

†**Pulmonary scoring** should be performed using both the symptom and pulmonary function testing (PFT) scale whenever possible. When discrepancy exists between pulmonary symptom or PFT scores the higher value should be used for final scoring. Scoring using the Lung Function Score (LFS) is preferred, but if DLCO is not available, grading using FEV1 should be used. The LFS is a global assessment of lung function after the diagnosis of bronchiolitis obliterans has already been established. The percent predicted FEV1 and DLCO (adjusted for hematocrit but not alveolar volume) should be converted to a numeric score as follows: >80% = 1; 70-79% = 2; 60-69% = 3; 50-59% = 4; 40-49% = 5; <40% = 6. The LFS = FEV1 score + DLCO score, with a possible range of 2-12.

Diagnosis and classification for chronic GvHD

Diagnosis of cGVHD according to NIH	
1) Presence of 1 diagnostic finding	
2) Presence of 1 distinctive finding confirmed by histological, radiological or any other investigation (e.g. Schirmer test), excluding any other potential cause	
Classification of cGVHD according to NIH	
1) Mild:	≤ 2 organ locations (lung excluded), maximal organ score 1
2) Moderate:	≥ 1 organ locations, maximal organ score 2 ≥ 3 organ locations, maximal organ score 1 Lung location score 1
3) Severe:	Organ score 3 in any organ Lung location score ≥ 2

1. Filipovich AH, Weisdorf D, Pavletic S et al. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Group Report. Biology of Blood and Marrow Transplantation 2005;11:945-956.
2. Glucksberg H, Storb R, Fefer A et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974;18:295-304.
3. Przepiorka D, Weisdorf D, Martin P et al. 1994 Consensus Conference on Acute GvHD Grading. Bone Marrow Transplant 1995;15:825-828.

J. Acute GVHD response definition

Complete response:	the return of acute GVHD to grade 0
Partial response:	improvement of at least 1 organ, with no worsening in other organs
Mixed response:	improvement of at least 1 organ, with worsening in at least 1 other organ
Stable disease:	no significant change in any organ system
Progressive disease:	progression in at least 1 organ system without improvement in any other organs